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Physiological quality in seeds of the common bean is affected by the period of nutri-priming[1]

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ABSTRACT - Nutri-priming is a low-cost and highly effective way of supplying seeds with micronutrients. The aim of this study was to evaluate the physiological quality of seeds of the Pérola cultivar of the common bean under nutrient solutions containing molybdenum, cobalt and zinc for different imbibition times. The experiment was carried out in the laboratory and greenhouse of the State University of Goiás UEG – UnU Ipameri, using a completely randomised experimental design, with eight micronutrient treatments (T1. Control; T2. Mo; T3. Zn; T4. Co; T5. Mo + Co; T6. Mo + Zn; T7. Zn + Co; T8. Mo + Co + Zn) and six imbibition times (6, 8, 12, 16, 20 and 24 hours). The variables under analysis were seed imbibition, germination test, first germination count, accelerated ageing, seedling emergence, emergence speed index, shoot length, root length, total length, fresh weight, dry weight, and shoot and root biomass. The micronutrients afforded an increase in germination in the seed batches under evaluation; on the other hand, imbibition times of greater than four hours reduced the germination capacity of the seeds. It can be concluded that nutri-priming in the common bean is only beneficial for an imbibition time of up to four hours.

Key words: Hydro-conditioning. Micronutrients. Germination. *Phaseolus vulgaris* L. Vigour.

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INTRODUCTION

The bean is a very important foodstuff, especially in developing countries, as it is an important source of energy with a low fat content (FAOSTAT, 2019). In large enterprises that are aimed at large-scale bean production, good results can be linked to the considerable amount of research that seeks to improve techniques of grain cultivation, such as direct planting, minimum tillage, integrated production, the choice of varieties adapted to a region, and quality seed production and treatment (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA, 2018).

The use of quality seeds is important when planting any crop, as they provide a stand that is appropriate to the plants (HAESBAERT *et al*., 2017); however, several factors can influence seed quality, including adequate plant nutrition during crop management. Plants with a nutrient deficiency produce seeds of inferior quality, altering germination potential and seed vigour, and affecting formation of the embryo and cotyledons (SILVA; OLIVEIRA; PEREIRA, 2018).

Nutrient-deficient soils require a large investment in liming and fertilisation, which is most often restricted to the use of NPK fertiliser, with producers and professionals in the area generally neglecting the use of micronutrients (BATISTA *et al*., 2018). Micronutrients are extremely important for the growth and quality of crops, and their deficiency can cause severe damage to plants. In most soils, micronutrient absorption can be affected by the low availability of organic matter or a high rate of application of phosphate fertiliser, in addition to such factors as pH, soil texture, the levels of iron oxide, aluminium and manganese, and the concentration and kind of constituents in the soil solution and their rate of percolation (SAFYAN; ABOUTALEBIAN; BAHARI, 2012).

Methods used to supply nutrients to the plants need further improvement to make better use of the applied nutrients and reduce costs to the producer (BERNARD *et al*., 2015). Treating seeds with micronutrients is a viable alternative for reducing the cost of purchasing inputs, their application and transport, since this technique can be more efficient than applying the nutrients directly to the soil − the uniformity of application to the seeds resulting in a larger area of contact between the nutrient and seed (SILVA; OLIVEIRA, 2021). According to Farooq, Wahid and Siddique (2012), the benefits of treating the seeds are even greater when it comes to their physiological quality. For those authors, treating the seeds with micronutrients, in addition to meeting the nutritional demand of the crop, improves seedling emergence, establishment, productivity and enrichment of the produced grains, making them more nutritious.

According to Bhowmick (2018), there are several ways of preparing seeds for a more uniform germination of greater quality using the mechanism of seed imbibition. The priming technique, developed by Higgins and Turner (1975) and optimised by Heydecker and Coolbear (1977), makes use of controlled hydration, with the aim of preparing the seed metabolism for germination. Use of priming together with the supply of nutrients is known as nutri-priming; this consists in soaking the seeds in a nutrient solution for a determined period before planting. This technique has shown potential effect in increasing the nutrient content of the seeds and improving germination (FAROOQ *et al*., 2011; SHIVAY *et al*., 2016).

Treating seeds with micronutrients can therefore be very effective in increasing bean production in Brazil. However, the literature still differs regarding the way nutrients should be applied to the seeds, whether individually or combined, and the positive or negative effects that treating the seeds can have on their physiological quality. The aim of this study, therefore, was to evaluate the physiological quality of bean seeds subjected to a nutrient solution (nutri-priming) containing Mo, Co and Zn, individually and combined, as a function of the imbibition time.

MATERIAL AND METHODS

Location of the experiment

The experiment was conducted at the Seed Laboratory (LASEM) and greenhouse of the State University of Goiás – Unu Ipameri, at 17º43' S and 48º09' W and an average altitude of 820 m. The climate in the region is classified as Aw, with an average annual precipitation and temperature of 1750 mm and 25 ºC, respectively. The study was carried out between August and September 2019.

Experimental design and treatments

The experimental design was completely randomised in an 8 x 6 factorial scheme, with four repetitions, each of 50 seeds, where the first factor refers to the combinations of micronutrients: T1. Control; T2. Mo; T3. Zn; T4. Dog; T5. Mo + Co; T6. Mo + Zn; T7. Zn + Co; T8. Mo + Co + Zn, in doses of 10 g zinc sulphate 10 kg^{-1} seeds; 5 g sodium molybdate 10 kg^{-1} seeds, and 2 g cobalt sulphate 10 kg^{-1} seeds, based on a study carried out by Smiderle *et al*. (2008). The second factor applies to the times of seed immersion in the nutrient solution (4, 8, 12, 16, 20 and 24 hours). The seeds used in the experiment came from the Pérola cultivar, part of the Carioca commercial group.

Evaluating seed imbibition

Seed imbibition was evaluated using four subsamples of 50 seeds, giving a total of 200 seeds per treatment, which were immersed in 600 mL of distilled water or in the micronutrient solutions, and kept at 25 ºC for 4, 8, 12, 16, 20 and 24 hours. After each period, the surface of the seeds was dried with paper towels and the seeds weighed on a digital balance to three decimal places. To evaluate the amount of water absorbed by the seeds, the moisture content and initial weight of the sample were determined using the oven method at 105 ± 3 ºC for 24 hours, as per the Rules for Seed Analysis (BRASIL, 2009), with the results expressed as percentage water.

Evaluating the physiological potential of the seeds

The parameters used to evaluate the physiological potential of the treated seeds were the germination test, first germination count, accelerated ageing, seedling emergence, emergence speed index, root length, shoot length and total length, as well as the fresh and dry weight of the roots and shoots, and total fresh and dry weight of the seedlings.

Germination Test: carried out on four repetitions of 50 seeds for each treatment, using a Paper Roll (PR) as substrate, moistened with deionised water at 2.5 times the dry weight of the paper; the seeds were then placed in a BOD chamber at 25 °C ± 1 °C. Evaluation followed the recommendations of Brasil (2009), where on the ninth day after setting up the test, the number of normal and abnormal seedlings and dead seeds were determined, with the results expressed as a percentage.

First Germination Count: conducted together with the germination test, considering the percentage of germinated seeds on the fifth day after setting up the experiment (BRASIL, 2009).

Accelerated Ageing: using four repetitions of 50 seeds from each treatment, which were spread over the surface of a metallic screen fixed and suspended inside a plastic gerbox containing 40 mL of deionised water, and kept at 4 °C for 72 h in a BOD chamber. The seeds were then removed from the chamber and left to germinate under the same conditions as the germination test. The normal seedlings and dead seeds were evaluated on the ninth day after setting up the test, with the results expressed as a percentage (%) (BRASIL, 2009).

Seedling Emergence: carried out on four repetitions of 50 seeds from each treatment, in plastic trays containing 6 kg of washed sand, sterilised in an autoclave for 2 hours; the seeds were sown at a depth of 3 cm, maintaining the humidity of the substrate at 60% of the retention capacity (BRASIL, 2009), and the trays kept in the greenhouse until the ninth day after installation. The number of normal emerged seedlings was determined, and the results expressed as a percentage.

Emergence Speed Index: carried out together with the seedling emergence test, where the number of seedlings with a visible cotyledon loop was recorded at the same time daily; at the end of the test, the index was calculated using the formula proposed by Maguire (1962).

Shoot, root and total length: the normal seedlings obtained in the emergence test were evaluated for shoot length, root length and total length with the aid of a ruler, and the results expressed in centimetres (NAKAGAWA, 1999).

Fresh weight, dry weight, and shoot and root biomass: the normal seedlings from each repetition were separated into shoots and roots and later weighed to obtain the fresh weight. The parts were then placed in paper bags and left to dry in a forced air circulation oven at 65 °C for 72 h; the samples were then left to cool in desiccators, and weighed on a precision balance, with the results expressed in mg plant^{-1}.

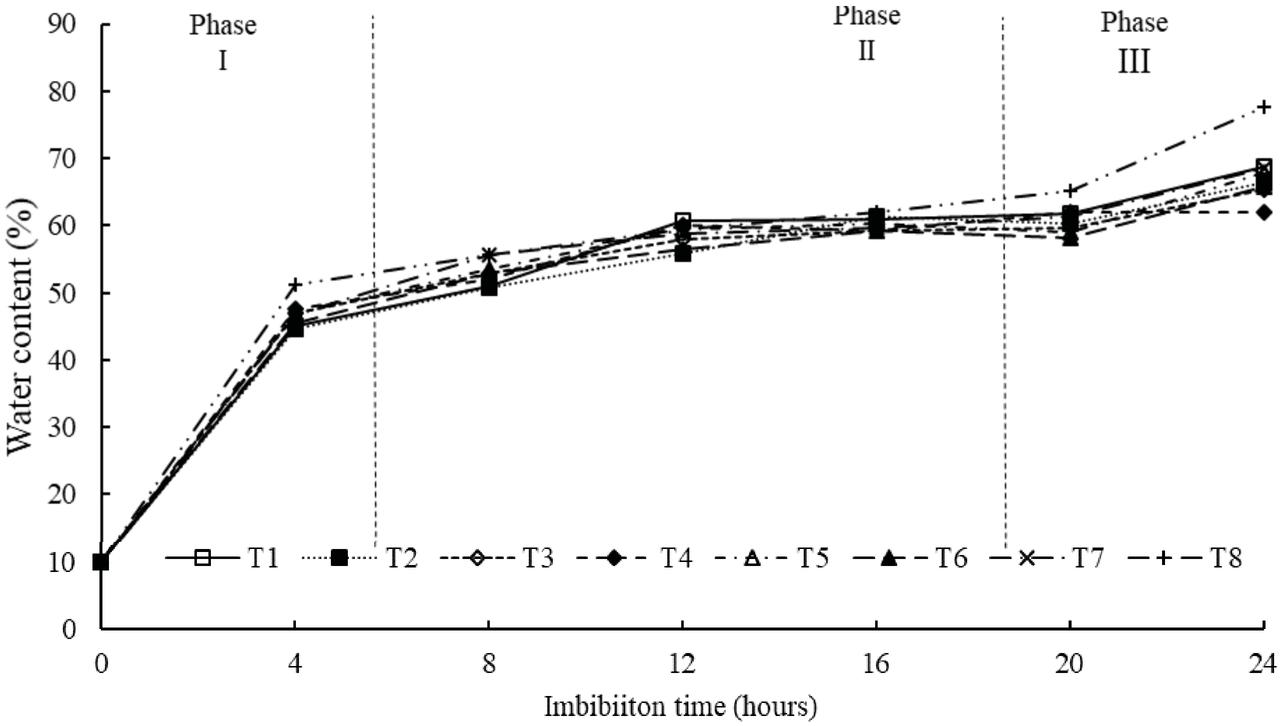
Statistical analysis

All the results of the parameters under evaluation were submitted to analysis of variance (ANOVA). When the isolated factor, the micronutrient combinations, showed significant results, the mean values were compared by Tukey's test at 5% probability. The imbibition times were submitted to regression analysis, with or without significant interaction between the factors. The software used in the analyses were the SISVAR 5.3 (FERREIRA, 2011) and SigmaPlot 10.0 (SYSTAT SOFTWARE, 2006).

RESULTS AND DISCUSSION

The initial water content of the seed batch was 9.97%. An average moisture gain of 52.88% was seen during the first eight hours of immersion (Figure 1). This increase is related to phase I of the germination process, when water is rapidly absorbed by the seed. Between 12 and 20 hours, there was slight stabilisation in the process of water absorption; at that time, the average moisture level of the seeds was 59.94%. This stabilisation is characterised as phase II of germination. During the next phase, the seeds again quickly absorb water, jumping to values of 67.18%; this increase is related to the start of phase III. Water absorption by the seeds is extremely important for germination, since during this process the seed tissue, which until then was dehydrated due to natural factors of the production phase, begin to re-hydrate, activating the seed metabolism and triggering germination (BASKIN; BASKIN, 2014).

Figure 1 - Imbibition curve in bean seeds as a function of combinations of molybdenum, cobalt and zinc for different imbibition times



T1 = Control; T2 = Molybdenum; T3 = Zinc; T4 = Cobalt; T5 = Molybdenum + Cobalt; T6 = Molybdenum + Zinc; T7 = Zinc + Cobalt; T8 = Molybdenum + Cobalt + Zinc

The results obtained for seed imbibition agree with those of Marcos-Filho (2015), who states that during the first phase of the imbibition process, seeds with cotyledonary reserves achieve a water content greater than 45%. During the first four hours of imbibition, it was possible to find bean seeds in all the treatments with levels equal to or greater than those reported by the above author. The water content continued to increase until around 12 hours imbibition. This rapid absorption is linked to the difference between the water potential of the solution and that of the seed.

During the second phase, which occurred between 12 and 20 hours after the start of imbibition, the speed of water absorption decreased; Marcos-Filho (2015) states that during this period, the moisture levels of the seeds stabilise, and respiration is drastically reduced. It is also at this time that fundamental metabolic processes take place for germination to continue (TAIZ; ZEIGER, 2017). In an experiment carried out by Smiderle *et al*. (2008), where they evaluated the rate of water absorption in seeds of the 'Pérola' bean, the authors also found the same period for phase II reported in the present experiment.

The resumption of water absorption seen after 20 hours from the start of imbibition ends with the protrusion of the primary root, completing the seed germination process (MARCOS-FILHO, 2015); however, the seeds were removed from the nutrient solution before protrusion occurred. During phase III, the nutrients contained in the cotyledons become more available for uptake by the embryonic axis, allowing for rapid establishment of the seedlings (ALI; ELOZEIRI, 2017). As reported by Carvalho and Nakagaw (2012), the increase in imbibition due to the demand of the seed and seedling at this point in the germination process increases growth, forming new cells, with the meristematic system absorbing the greatest amount of water. For several researchers, obtaining the water absorption curve of the seeds is of great importance, since, referring to non-dormant viable seeds, germination begins with the imbibition of water, and triggers a series of metabolic changes that lead to formation of the primary root (BRADFORD, 1995; CARVALHO; NAKAGAWA, 2000; EIRA; CALDAS, 2000; LABOURIAU, 1983).

The results of the analysis of variance for the first germination count (FGC), germination test (G), abnormal seedlings (AS), dead deeds (DS), accelerated ageing (AA), dead seeds from accelerated ageing (DSAA), and emergence in the greenhouse (EGH), revealed a significant effect from the interaction of the treatments with the micronutrients and imbibition times, with the exception of the Speed of Emergence Index (SEI). With FGC, the data referring to the imbibition time adjusted to a quadratic regression for T1 (control), and to a linear regression for the other treatments (Figure 2). It can be seen that for T1, a period close to 12 hours gave the best results for FGC, whereas for the other treatments, the values decreased linearly.

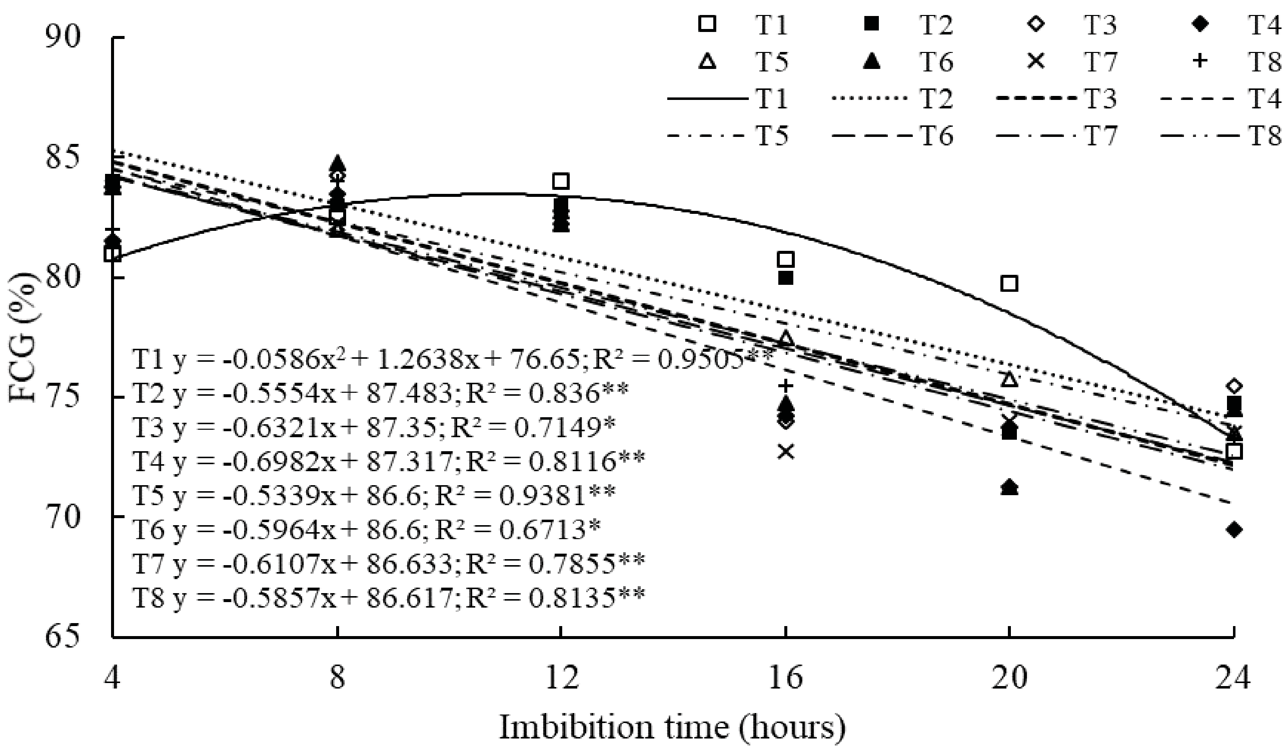
The First Germination Count test (FGC) showed a significant difference only between the control treatment (T1) and the nutrient solution containing Co (T4); the remaining treatments did not differ. FGC is a test carried out to estimate the percentage of germinated seeds on the fifth day after setting up the germination test (BRASIL, 2009), batches with greater vigour germinate more quickly, favouring seedling establishment in the soil, and leaving them less prone to abiotic stress (MARCOS-FILHO, 2015). Based on the results for FGC, a reduction of 3.22% can be seen in the germination rate of the seed batch submitted to the nutrient solution containing Co (Figure 2).

Cobalt is a very important micronutrient in biological nitrogen fixation (ALVES *et al*., 2018), and when applied directly to the seeds of plants from family Fabaceae, has been shown to promote satisfactory results in seed germination (GUERRA; MEDEIROS FILHO; GALLÃO, 2006). However, the same result was not found in the present study, where the total number of germinated plants on the fifth day was lower than that of the control treatment (T1), confirming Smiderle *et al*. (2008) and Golo *et al*. (2009), who claim that cobalt does not exert a great influence on the seed germination process.

When evaluating germination, a significant interaction was seen for all treatments, with the negative linear regression model best representing the data. As a result, there was an average reduction of 23.5% in the total number of normal seedlings at the end of the germination test (Figure 3). An increase in imbibition time led to an increase in the number of abnormal seedlings and dead seeds, of 7.25% and 19%, respectively, as shown in Figures 4 and 5.

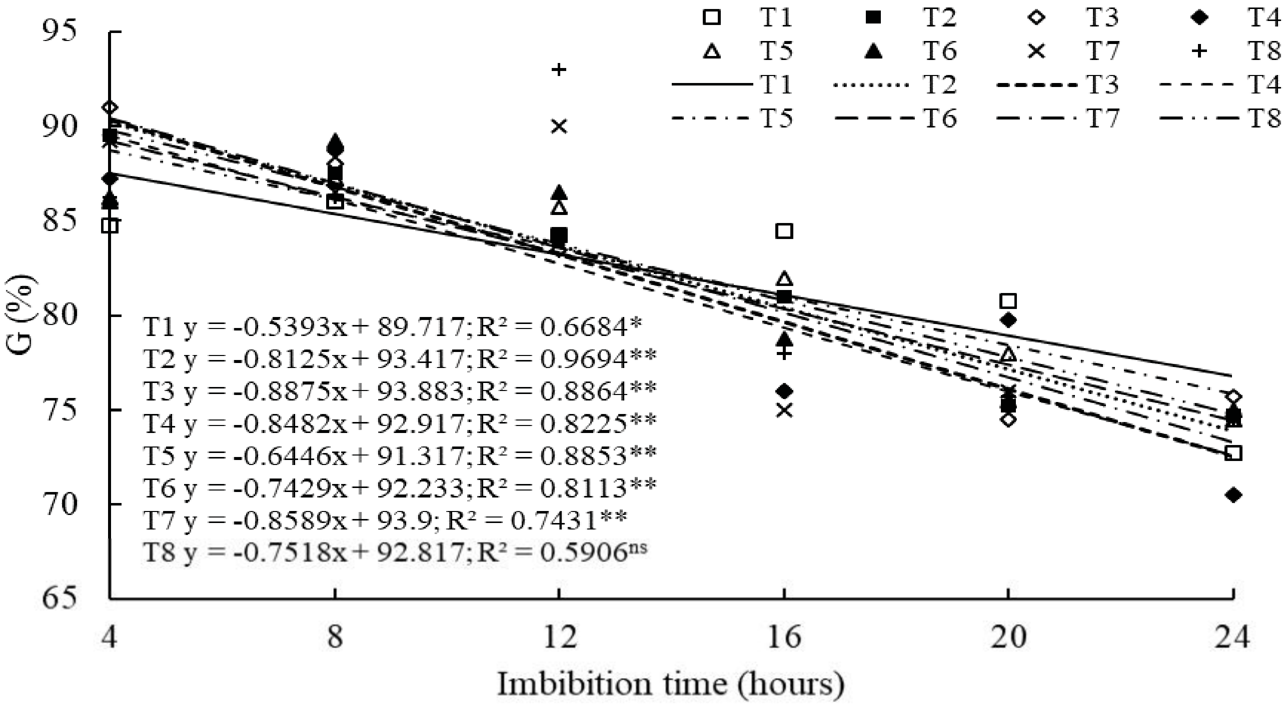
The increase in the percentage of dead seeds, and the consequent reduction in total germination values as a function of imbibition time, is related to the low tolerance of the seeds to prolonged periods of imbibition; in a study by Custódio *et al*. (2002), immersing bean seeds in water for periods greater than 24 hours promoted an increase of 80% in the number of dead seeds.

Figure 2 - First germination count as a function of combinations of molybdenum, cobalt and zinc for different imbibition times. *significant at 5% probability; **significant at 1% probability



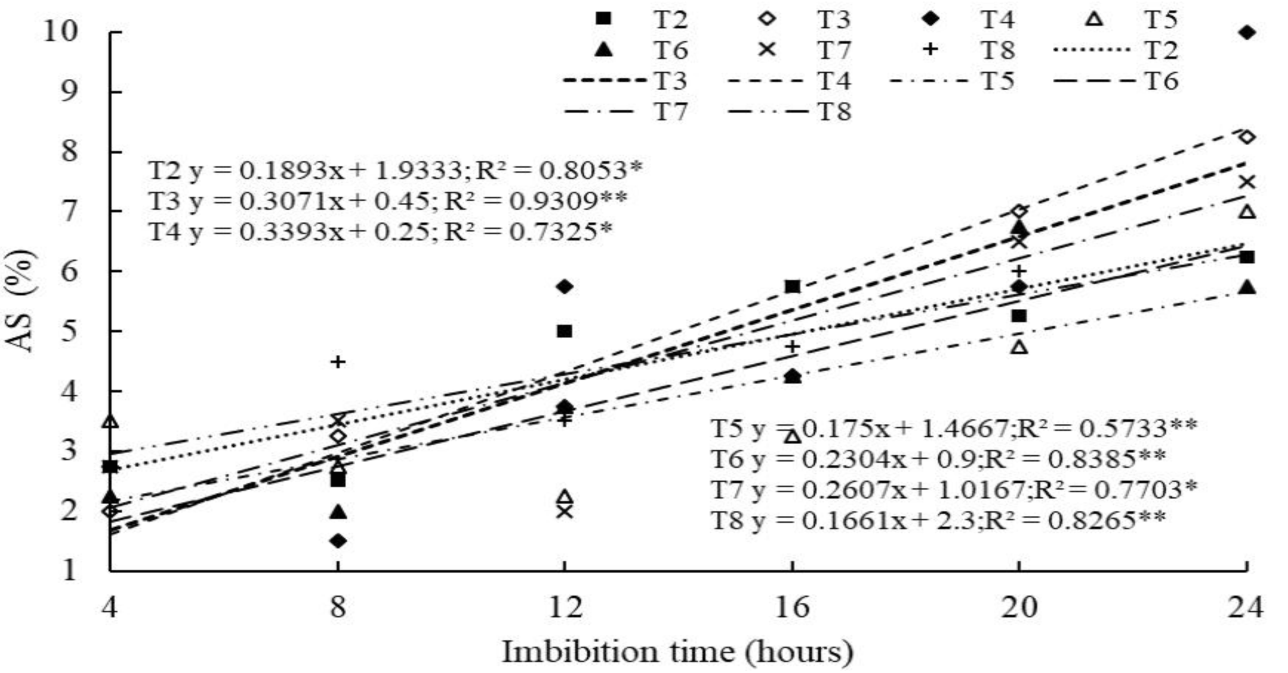
T1 = Control; T2 = Molybdenum; T3 = Zinc; T4 = Cobalt; T5 = Molybdenum + Cobalt; T6 = Molybdenum + Zinc; T7 = Zinc + Cobalt; T8 = Molybdenum + Cobalt + Zinc

Figure 3 - Germination as a function of combinations of molybdenum, cobalt and zinc for different imbibition times. *significant at 5% probability; **significant at 1% probability



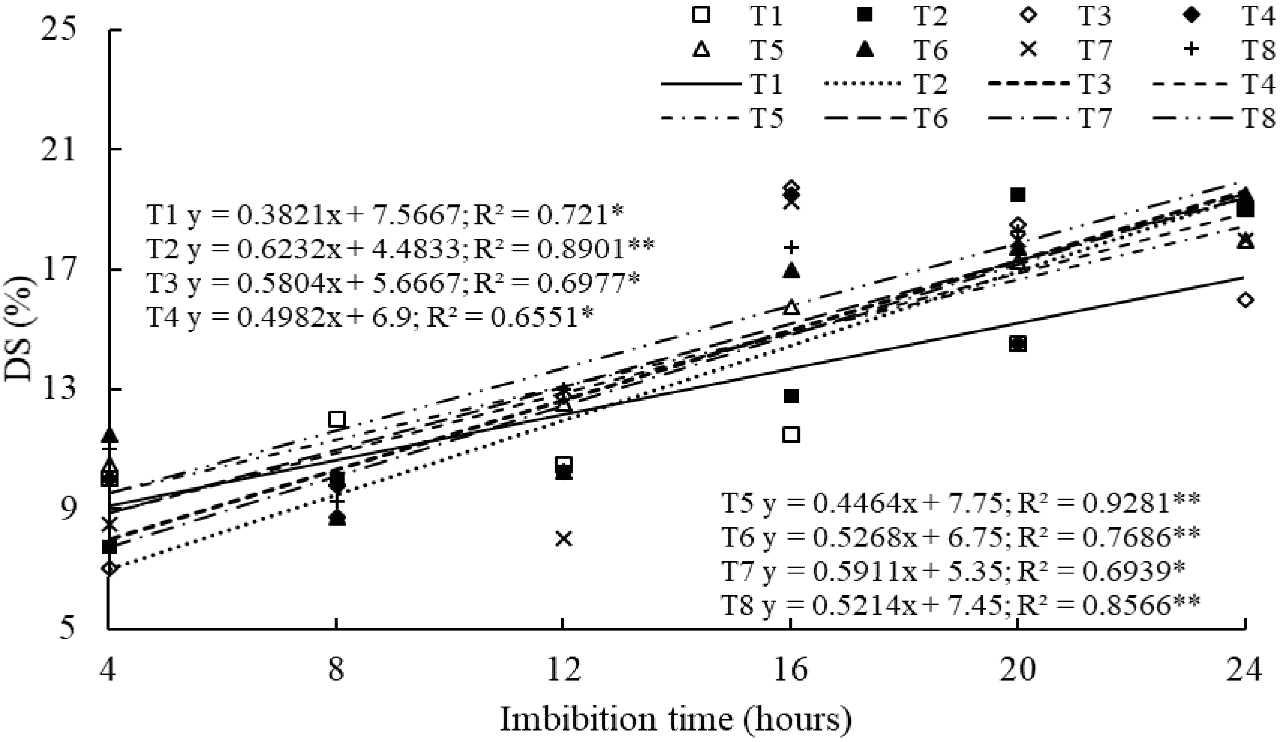
T1 = Control; T2 = Molybdenum; T3 = Zinc; T4 = Cobalt; T5 = Molybdenum + Cobalt; T6 = Molybdenum + Zinc; T7 = Zinc + Cobalt; T8 = Molybdenum + Cobalt + Zinc

Figure 4 - Abnormal seedlings as a function of combinations of molybdenum, cobalt and zinc for different imbibition times. *significant at 5% probability; **significant at 1% probability



T1 = Control; T2 = Molybdenum; T3 = Zinc; T4 = Cobalt; T5 = Molybdenum + Cobalt; T6 = Molybdenum + Zinc; T7 = Zinc + Cobalt; T8 = Molybdenum + Cobalt + Zinc

Figure 5 - Dead seeds as a function of combinations of molybdenum, cobalt and zinc for different imbibition times. *significant at 5% probability; **significant at 1% probability



T1 = Control; T2 = Molybdenum; T3 = Zinc; T4 = Cobalt; T5 = Molybdenum + Cobalt; T6 = Molybdenum + Zinc; T7 = Zinc + Cobalt; T8 = Molybdenum + Cobalt + Zinc

The reduction in value of the parameters under evaluation for germination, and the increase in the amount of dead seeds and abnormal seedlings, may be related to the lack of oxygen to which the seeds were subjected over a long period, causing hypoxia, and transforming respiration into fermentation, resulting in the generation of ethanol or lactate, both of which are toxic substances to the respiratory metabolism, in addition to reducing ATP synthesis (OGAWA *et al*., 2016). When seeds are subjected to rapid imbibition, damage occurs in restructuring the cell membranes, promoting the leaching of cell contents that are important to the germination process (CASTRO; HILHORST, 2004).

With accelerated ageing (Figure 6), there was a linear reduction in germination for the seeds under analysis, leading to a linear increase in the number of dead seeds (Figure 7). It can also be seen that only T5 adjusted to the regression, the other treatments not showing a significant trendline.

The use of the accelerated ageing test to evaluate the behaviour of a batch of seeds by subjecting them to stress reduces the percentage of germination. In the present study, it was found that for imbibition times between 4 and 12 hours the seeds continued to present values for germination above 80% (Figure 6). These results are superior to those found by Smiderle *et al*. (2008) in a similar experiment, where the percentage of germination was less than 59%.

For the SEI, as there was no significant difference in the interaction between factors, the data were evaluated using Tukey's test for both the micronutrient combinations and the imbibition time (Table 1). Treatments T3, T5, T6, T7 and T8 presented the best results for the SEI. These treatments, with the exception of T5, included Zn in the nutrient solution, which increased the germination rate by 25% compared to the control treatment. It can also be seen that an imbibition time of 12 hours gave the best result for the SEI; this is similar to the FGC data, where a time of 12 hours for T1 afforded the best responses.

According to Oliveira *et al*. (2014), seeds from places where the soil is poor in zinc contain low levels of the nutrient, resulting in a reduction in vigour for the batch. Applying zinc via the seeds, in addition to offering reduced production costs by avoiding application via the leaf, also improves seedling establishment, which at this stage have a limited root system and shoots that are inefficient at foliar absorption (TUNES *et al*., 2012).

Figure 6 - Accelerated ageing as a function of combinations of molybdenum, cobalt and zinc for different imbibition times. *significant at 5% probability; **significant at 1% probability

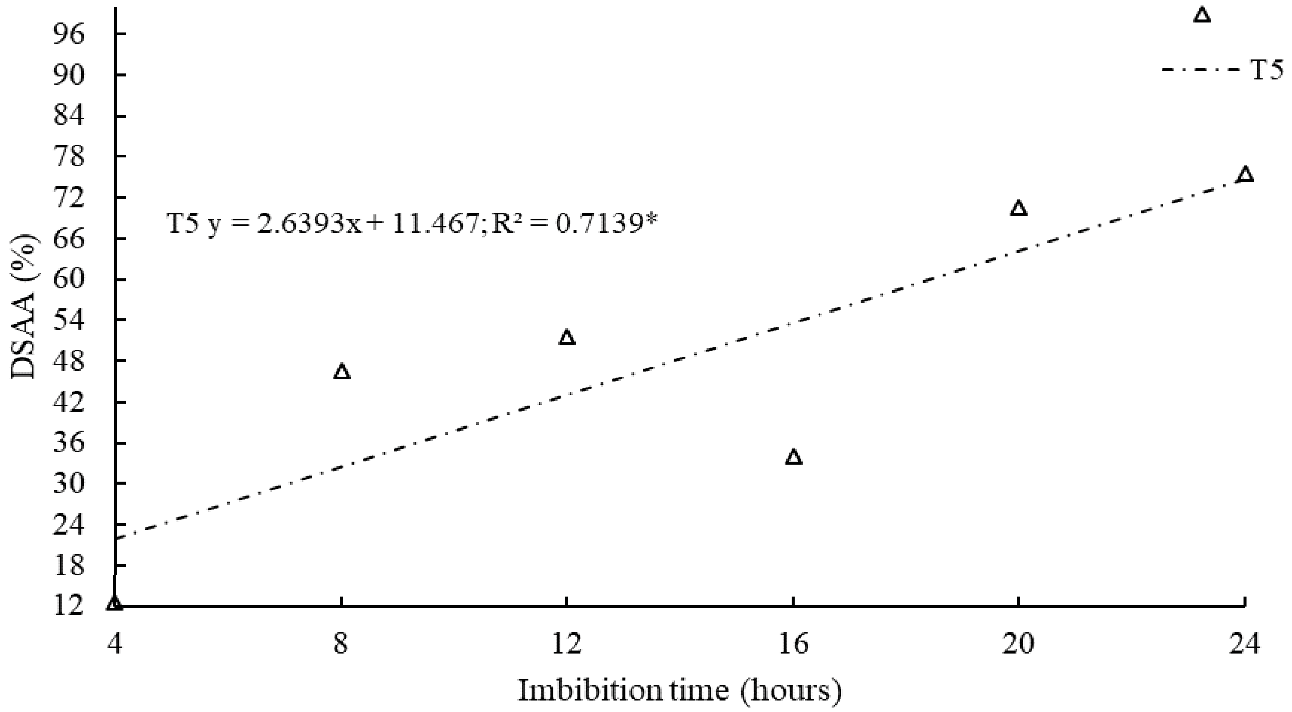
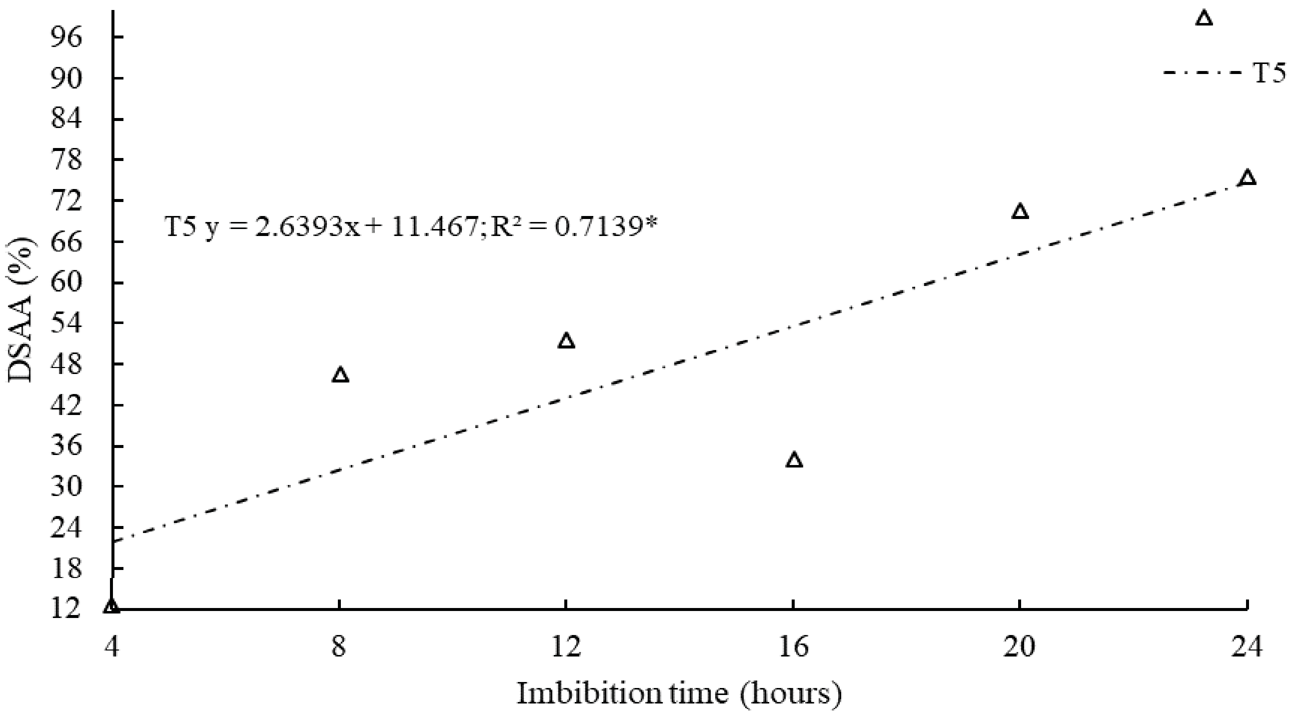


Figure 7 - Dead seeds from accelerated aging as a function of combinations of molybdenum, cobalt and zinc for different imbibition times. *significant at 5% probability; **significant at 1% probability



In addition to zinc, molybdenum also contributed to better a SEI and FE index. The micronutrient is directly related to several enzymes, including nitrate reductase, whose deficiency can reduce the capacity for biological nitrogen fixation and affect crop productivity (TAIZ; ZEIGER, 2017). The application of

Mo to sorghum seeds, with a view to increasing the their physiological quality, promoted higher values for FGC and SEI. This suggests that the micronutrient is beneficial for initial seedling development (CUNHA *et al*., 2015). In supplying Mo via the seeds, Arun *et al*. (2017) also observed significant improvements in cowpea germination, corroborating the responses obtained in the present study.

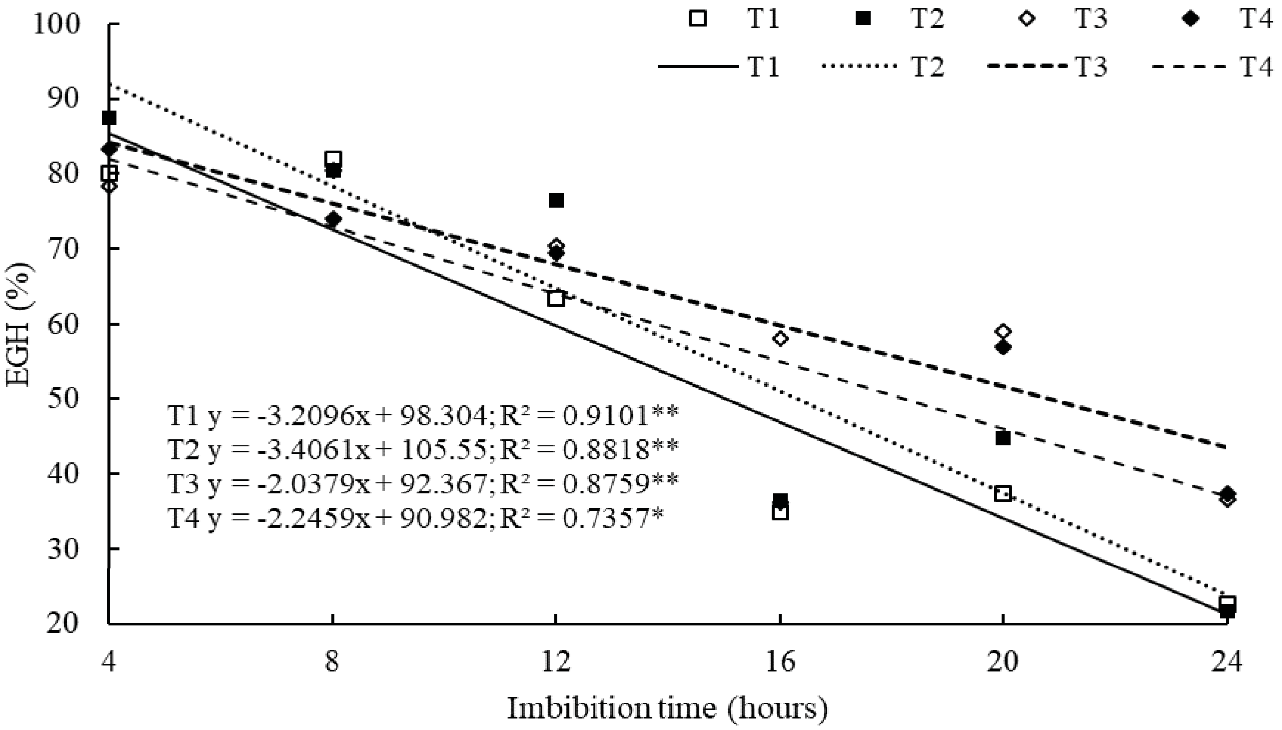
For the test of emergence in the greenhouse (EGH) (Figure 8), increasing the imbibition time reduced the rate of seedling emergence. This result is in line with those found by Custódio *et al*. (2009) and Ogawa *et al*. (2016), where longer imbibition times caused a significant fall in the values obtained for the variables. The results for EGH are confirmed by those obtained in the laboratory, however, it is worth noting that in an experiment by Santos-Moura *et al*. (2019), where they evaluated the behaviour of bean seeds subjected to increasing imbibition times, they found a growing improvement in emergence variables up to a period of five hours imbibition.

Table 1 - Mean values for the Speed of Emergence Index (SEI) as a function of micronutrient combinations and imbibition time

Speed of emergence index			
Micronutrient combination (Mo, Zn and Co)		Imbibition time (hours)	
T1 (Control)	4.26 d	4	4.37 cd
T2 (Mo)	4.71 cd	8	4.67 cd
T3 (Zn)	5.36 ab	12	7.52 a
T4 (Co)	4.85 bcd	16	4.01 d
T5 (Mo + Co)	5.57 a	20	6.36 b
T6 (Mo + Zn)	5.49 a	24	3.63 d
T7 (Zn + Co)	5.19 abc		
T8 (Zn + Co + Mo)	5.74 a		

Mean values followed by the same letter in a column do not differ by Tukey's test at 5% probability

Figure 8 - Emergence in the greenhouse (EGH) as a function of combinations of molybdenum, cobalt and zinc for different imbibition times. *significant at 5% probability; **significant at 1% probability



T1 = Control; T2 = Molybdenum; T3 = Zinc; T4 = Cobalt; T5 = Molybdenum + Cobalt; T6 = Molybdenum + Zinc; T7 = Zinc + Cobalt; T8 = Molybdenum + Cobalt + Zinc

There was a significant difference between the isolated factors for the variables, shoot length (SL), root length (RL), total length (TL), shoot fresh weight (SFW), root fresh weight (RFW), total fresh weight (TFW), shoot dry weight (SDW), root dry weight (RDW) and biomass (BIO). For SL, treatment T4 showed the best result, albeit not differing from T7 or T3, with a hypocotyl length greater than 6 cm. Treatments T7, T5 and T3 resulted in seedlings of similar root length. In general, TL followed the trend of the other two variables, since total seedling length is obtained by summing SL and RL (Table 2).

According to Nakagawa (1999), it is extremely important to evaluate seedling length, given that batches with greater seedling length become better established, absorbing a larger amount of nutrients and more sunlight, giving rise to more vigorous plants that are less prone to competition from weeds.

The variables of length (SL, RL, TL) benefitted from the presence of zinc and cobalt. According to Rouhi, Aboutalebian and Sharif-Zadeh (2011), applying Zn to the seeds improves the mean length of the shoots and roots of the seedlings, since Zn induces several metabolic activities whose product is used in protein synthesis, increasing the germination rate and leading to an initial uniform stand of seedlings (IMRAN; BOELT; MÜHLING, 2018).

In a study by Marchetti *et al*. (2006), a supply of Co resulted in an improvement in the initial development of soybean seedlings, corroborating the results of the present study. However, several authors cite this nutrient as being far more important during the process of biological nitrogen fixation than during the initial process of germination and seedling establishment (MARCARELLO; YANASHITA; CARVALHO, 2012; RAIJ, 1991).

All the treatments showed better results for fresh weight (SFW, RFW and TFW) than the control, with the exception of the combination of Mo and Zn (T6), which showed the lowest values for these variables (Table 3). It should be noted that, individually, these micronutrients did not differ significantly from each other, and that the reduction in growth rates may be related to some toxic effect caused by the combination of micronutrients. Similar results were obtained for dry weight (SDW, RDW and BIO).

As with the variables of length, seedlings with a higher fresh-weight content are less prone to competition from weeds. For the root system, Hartwigsen and Evans (2000) report that seedlings with greater root fresh weight are able to produce a more robust rhizosphere, leading to an improvement in water and nutrient absorption, and leaving the seedlings less prone to stress.

Among the micronutrients used in this experiment, Zn proved to be one of the most beneficial. According to Sharma and Agrawal (2005), appropriate levels of zinc can promote an increase in the photosynthetic area of plants, resulting in increased fresh weight and biomass. Dörr *et al.* (2018) report that applying Zn to bean seeds had a positive effect on their physiological quality, promoting superior shoot and root growth in the seedlings compared to the control.

Mo also showed satisfactory results, whether applied individually or combined with Co. A similar result was found by Santos-Moura *et al.* (2019), where the application of Mo promoted an increase in the biomass of bean seedlings. The variables for seedling dry weight present important data, as they show the transfer of reserves contained in the seeds to the embryonic axis during the germination phase, demonstrating that seedlings that receive a greater amount of nutrients accumulate more biomass.

Table 2 - Mean values for shoot length (SL), root length (RL) and total length (TL) as a function of combinations of molybdenum, cobalt and zinc

Micronutrient combination	SL	RL	TL
	---------- cm ----------		
T1 (Control)	5.51 cde	3.79 c	9.30 de
T2 (Mo)	5.97 bc	4.20 bc	9.99 bcd
T3 (Zn)	6.16 ab	4.29 ab	10.46 abc
T4 (Co)	6.53 a	4.21 bc	10.75 ab
T5 (Mo + Co)	5.79 bcd	4.32 ab	10.11 abcd
T6 (Mo + Zn)	5.16 e	3.89 bc	9.05 e
T7 (Zn + Co)	6.18 ab	4.71 a	10.89 a
T8 (Zn + Co + Mo)	5.47 de	4.21 bc	9.69 cde

Mean values followed by the same letter in a column do not differ by Tukey’s test at 5% probability

The physiological conditioning of seeds has been widely used to standardise the germination of crops of agricultural interest. The direct immersion of seeds in water is a low-cost technique; however, as seen in this study, it has a negative impact on germination. Further studies, to investigate slower water absorption by seeds, are therefore necessary.

The data for SL, RL and TL did not adjust to the models for imbibition time, so the variables were evaluated using Tukey's test. The best results are seen for the imbibition time of 4 hours, with seedling lengths greater than 13 cm (Table 4). As for imbibition time and the variables of weight (SFW, RFW, TFW, SDW, RDW and BIO), the regression did not adjust to any model, with the mean values being compared using Tukey's test. For all the variables, the best imbibition time was four hours, with the exception of BIO, where the period of twenty hours showed no significant difference to that of four hours (Table 5).

These results are similar to those of Santos-Moura *et al*. (2019), who found that the variables of length showed higher values when the seeds were subjected to five hours imbibition; on the other hand, Ogawa *et al*. (2016) found a reduction in the length of bean seedlings for an imbibition time of between two and eight hours, with seedlings measuring between 6 and 7.5 cm.

It is important to emphasise that there was a marked reduction in the values of SL, RL and TL during the eight-hour period of imbibition, when the seeds were still in phase I of the germination process. Removing the seeds from the nutrient solution may have prevented the seeds from producing adequate levels of carbohydrates, proteins and lipids and, as a result, the reserve substances in the seeds were not broken down into smaller molecules, reducing the supply of nutrients to the embryonic axis (CARVALHO; NAKAGAWA, 2000).

Table 3 - Mean values for shoot fresh wet (SFW), root fresh weight (RFW), total fresh weight (TFW), shoot dry weight (SDW), root dry weight (RDW) and biomass (BIO) as a function of combinations of molybdenum, cobalt and zinc

Micronutrient combination	SFW	RFW	TFW	SDW	RDW	BIO
	---------- mg pl^{-1} ----------					
T1 (Control)	202.36 cd	141.82 bc	344.18 cd	14.67 c	9.91 d	24.59 d
T2 (Mo)	249.64 ab	167.88 a	417.53 ab	19.19 a	12.98 ab	32.17 a
T3 (Zn)	254.85 a	179.06 a	433.92 a	18.44 a	12.91 abc	31.36 ab
T4 (Co)	253.82 a	161.84 ab	415.66 ab	17.27 ab	10.56 d	27.84 bcd
T5 (Mo+Co)	216.14 c	164.92 a	381.07 bc	17.94 a	13.31 a	31.26 ab
T6 (Mo+Zn)	185.27 d	137.85 c	323.13 d	15.27 bc	11.39 bcd	26.66 cd
T7 (Zn+Co)	225.74 abc	163.95 a	389.70 abc	17.04 abc	12.22 abc	29.27 abc
T8 (Zn+Co+Mo)	221.87 bc	164.97 a	386.84 abc	14.83 bc	11.27 cd	26.10 cd

Mean values followed by the same letter in a column do not differ by Tukey's test at 5% probability

Table 4 – Tukey's test for shoot length (SL), root length (RL) and total length (TL) as a function of imbibition time

Imbibition time (hours)	SL	RL	TL
	---------- cm ----------		
4	7.69 a	6.72 a	14.01 a
8	3.78 e	2.67 e	6.39 e
12	4.96 d	3.22 d	8.19 d
16	6.43 c	4.32 c	10.76 c
20	7.04 b	5.01 b	11.68 b
24	5.17 c	3.19 d	8.36 d

Mean values followed by the same letter in a column do not differ by Tukey's test at 5% probability

Table 5 – Tukey's test for shoot fresh weight (SFW), root fresh weight (RFW), total fresh weight (TFW), shoot dry weight (SDW), root dry weight (RDW) and biomass (BIO) as a function of imbibition time. Ipameri, Goiás, 2020

Imbibition time (hours)	SFW	RFW	TFW	SDW	RDW	BIO
	mg pl^{-1}					
4	344.84 a	284.18 a	627.95 a	21.14 a	17.73 a	38.88 a
8	125.66 e	102.62 d	249.44 e	11.42 d	8.04 e	19.46 d
12	179.14 d	119.48 d	304.45 d	11.57 d	7.51 e	19.08 d
16	236.53 c	156.89 c	391.44 c	18.85 b	12.53 c	31.38 b
20	265.64 b	186.30 b	451.09 b	21.88 a	15.17 b	37.06 a
24	182.19 d	112.24 d	294.95 d	16.14 c	9.95 d	26.09 c

Mean values followed by the same letter in a column do not differ by Tukey's test at 5% probability

The results of the present study confirm what was said by Custódio *et al*. (2002), who state that bean seeds do not respond well to long periods of imbibition. A similar result was found by Dantas *et al*. (2000), where long imbibition times caused harmful effects to initial seedling development. It is worth noting, however, that for periods of up to five hours, the results for the agronomic characteristics of the seeds were favourable.

The reductions seen for prolonged imbibition times may be related to rapid water absorption by the seeds. The above-mentioned authors report that when a seed rapidly absorbs water, there is not enough time to restructure the membranes and the cells are unable to return to a properly structured state, allowing leaching of the substances contained in the seeds that influence the germination process (CUSTÓDIO *et al*., 2002; DANTAS *et al*., 2000).

CONCLUSIONS

1. Supplying micronutrients using the technique of nutri-priming affords an increase in the physiological quality and vigour of the seeds, with treatments that include Zn giving the best results;
2. Imbibition times greater than four hours are detrimental to the characteristics of the seeds under evaluation.

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