RESEARCH NOTE

Performance of rice seeds treated with zinc, boron, and molybdenum[1]

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ABSTRACT - The objective of this study was to assess physiological quality of seeds and the initial growth of lowland rice seedlings, cv. PUITA INTA-CL, treated with a product based on Zinc, Boron and Molybdenum. The following dosages of product were used: 0 (control), 50, 100, 150 and 200 mL.100 kg^{-1} seed. A completely randomized experimental design was used, with 10 treatments and four replications. Physiological quality of seeds was assessed by following tests: germination; first count of germination; and length and dry phytomass of seedlings. The initial growth was assessed at 7, 14, 21, 35, and 42 days after emergence (DAE) through plant height, leaf area and dry phytomass of aerial parts and roots. It was concluded that the treatment of rice seeds with the product containing Zinc, Boron and Molybdenum up to the dosage tested of 200 mL.100 kg^{-1} seed does not influence germination, first count of germination, and the dry phytomass of aerial parts and roots; however, negatively influences seedling length. In addition, it also promotes greater plant height, higher leaf area, higher production of dry phytomass of seedlings, and increase on the growth rate, until 42 DAE.

Index terms: *Oryza sativa*, initial growth, physiological quality, growth rate.

Desempenho de sementes de arroz tratadas com zinco, boro e molibdênio

RESUMO – O objetivo deste estudo foi avaliar a qualidade fisiológica das sementes e o crescimento inicial das plântulas de arroz irrigado, cultivar PUITA-INTA CL, tratadas com um produto a base de zinco, boro e molibdênio. Foram testadas as seguintes doses do produto: 0 (testemunha), 50, 100, 150 e 200 mL.100 kg^{-1} de sementes. Foi utilizado o delineamento experimental inteiramente casualizado, com 10 tratamentos e quatro repetições. A qualidade fisiológica das sementes foi avaliada pelos seguintes testes: germinação; primeira contagem de germinação; e comprimento e fitomassa seca de plântulas. O crescimento inicial foi avaliado aos 7, 14, 21, 28, 35 e 42 dias após a emergência (DAE) pela altura de planta, área foliar e fitomassa seca de parte aérea e raiz. Concluiu-se que o tratamento de sementes de arroz irrigado com o composto contendo zinco, boro e molibdênio, até a dose estudada de 200 mL.100 kg^{-1} de semente, não influencia germinação, primeira contagem da germinação, fitomassa seca de parte aérea e raiz; entretanto influencia negativamente o comprimento das plântulas. Além disso, também promove maior altura de planta, maior área foliar, maior produção de fitomassa seca de planta e aumento da taxa de crescimento, até os 42 DAE.

Termos para indexação*: Oryza sativa*, crescimento inicial, qualidade fisiológica, taxa de crescimento.

Introduction

During the summer harvest of 2010/2011, the rice production in Brazil has been 12.6 million metric tons, and the State of Rio Grande do Sul, the southernmost Brazilian state, has produced 8.2 million metric tons; what is equivalent to 65% of the nationwide production and characterizes the state as the largest rice producer in the country (CONAB, 2011). In the last decades, studies concerning soil correction and lowland rice fertilization in the South Region of Brazil were intensified

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aiming at searching for more consistent information on response of lowland rice to sources, dosages, and application timing of Nitrogen, Phosphorus, Potassium, and some micronutrients. Up to moment, studies performed in the State of Rio Grande do Sul indicate that the soils adapted for lowland rice cultivation are generally well provided of micronutrients due to good responses observed in experiments (Scivittaro and Gomes, 2007).

Among the essential micronutrients to development of plants, the Boron (B) plays an important role on pollen germination and on pollen tube growth; events particularly important for production of crops. For that occur growth of the pollen tube, high concentrations of B on stigma and on style are needed for physiological inactivation of calluses, by means of borate-callose complexes formation on the interface of pollen tube/style (Lewis, 1980).

Zinc (Zn) is the micronutrient that most frequently is deficient in Brazilian soils. Within the cropping areas of rice in Rio Grande do Sul, where rice monoculture generally occurs for several years, and in consequence of: systematizations and flooding; successive cultivation of the areas and of other factors, such as cultivars nutritionally demanding, use of fertilizers formulations with high concentrations; and lack of soil acidity correction, makes that occur deficiency of micronutrients, among which the most affected is Zn (Marchesan et al., 2001; Silva et al., 2003).

The interest by study of using Molybdenum (Mo) on rice is recent and the available literature has been showing important and promising results with the application of this micronutrient; chiefly in the last 10 years (Sundim et al., 2002). There are studies reporting reduction on rice sterility percentage (Ambak and Tadano, 1991), increment on production of grains (Muralidharan and José, 1994); and results confirming the benefits of Mo application associated to increases on production of grains, when the element is used in association with Zn and Copper (Cu) (Rafey et al., 1992).

There are different forms of assessing effect of nutrients on vegetal growth. One of them is the growth analysis; what according to Campos et al. (2008), is an important manner for explaining plant growth, once circa 90% of dry mass accumulated within them, along their development are resulting from photosynthetic activity, thus allowing for an assessment of final growth as a whole, as well as the contribution of each of the different organs on the total development of the plants.

The nutritional demand for micronutrients by plants is low. Therefore, the seed treatment with Zn, B, and Mo rises as an excellent alternative to supply the nutritional needs of plants at initial developmental stages. The application of micronutrients via seeds may exempt other application forms; thus reducing production costs. It should be emphasized, however, that the studies already performed are still scarce and somehow insufficient. Thus, further studies are needed to confirm these preliminary assessments.

Therefore, the objective of this study was to assess physiological quality of seeds and initial growth of seedlings of lowland rice treated with Zn, B, and Mo.

Material and Methods

The study has been developed during the 2009/2010 agricultural harvest, in the Didactic Laboratory of Seed Analysis and under greenhouse conditions, at the Eliseu Maciel Agronomy College of the Federal University of Pelotas, municipality of Capão do Leão, State of Rio Grande do Sul, Southern Brazil. For the experiments, the lowland rice cultivar PUITA INTA-CL was used.

The seeds were treated with the commercial product Binova GRA®, formulated with Zn, B, and Mo in the following concentrations 1.23, 41.82, and 43.05 $g.L^{-1}$ of each element, respectively. The treatments have consisted in the following dosages of the commercial product: 0 (control), 50, 100, 150, and 200 $mL.kg^{-1}$ seed. The treatment of the seeds was performed according to methodology recommended by Nunes (2005), in which the micronutrients were placed directly at bottom of a transparent plastic bag until a height of approximately 15 cm. Soon after, 200 g of lowland rice seeds were added to the plastic bag, which was then shaken during three minutes. In sequence, the seeds were dried under room temperature, during 24 hours.

Aiming at assessing possible positive effects of the product on the physiological quality of seeds, the following characteristics were evaluated:

Germination (G): this test was performed using four replications of 50 seeds each, for each replication of the different treatments. For that, the seeds were evenly distributed on top of two sheets of Germitest® paper, covered with another sheet of the same paper, with all paper sheets previously moistened with distilled water in a volume equivalent to 2.5 times the mass of the dry substrate. The set (paper + seeds) was then transformed into a roll. Immediately after, the rolls containing seeds were placed into a seed germinator, at 25 ± 2 °C; and assessments were performed 14 days after sowing (DAS) (Brasil, 2009).

First count of germination (FCG): in this test was determined de percentage of normal seedlings and was carried out at seven DAS by using the germination test already described.

Total length of seedlings and lengths of aerial parts and roots (TLS, LAP, and LR): this assessment was performed by determining total length and length of aerial parts on 10 normal seedlings at the seventh DAS, with the aid of a ruler graduated in millimeters. The length of root was determined by subtracting the length of aerial part from the total length of each seedling assessed with results expressed in $cm.seedling^{-1}$.

Total dry phytomass of seedlings and dry phytomass of aerial parts and roots (TDP, DPAP, and DPR: such assessment was performed jointly with test where length of seedlings was determined. Aerial parts and roots were cut with the aid of a scalpel, placed into Kraft paper bags, and taken to dry in a forced air circulation drying oven, at 60 ± 2 °C, during 48 hours. After that period, the samples were placed to cool into desiccators, and were then weighed in a centesimal precision analytical balance. Results were expressed in $mg.seedling^{-1}$ (Nakagawa, 1999).

In addition to physiological quality assessment of seeds immediately after treatment, the sowing has been performed and the experiment was conducted until 42 DAS. For that, treated seeds were sown on seedling-beds (1.0 m x 6.0 m) filled with soil collected in A1 horizon of a "Planossolo Háplico eutrófico solódico" (according to the Brazilian soil classification) (Streck et al., 2008), belonging to the soil mapping unit of Pelotas. The fertilization was performed according to Commission of Soil Fertility and Soil Chemistry for the states of Rio Grande do Sul and Santa Catarina (CFQS - Comissão de Fertilidade e Química do Solo – RS/SC, 2004), incorporating the nutrients to soil at sowing moment.

The samplings for assessing rice seedlings growth were performed, and afterwards the following determinations were carried out: plant height (PH); leaf area (LA); and dry phytomass of aerial parts (DPAP). For these determinations, 10 plants per treatment were cut at the soil line at 7, 14, 21, 28, 35, and 42 DAE. The LA was determined by using a photoelectric leaf-area meter (brand Li-cor Ltd., model LI-3100), which provides direct reading in cm^2. For determining PH, the measurements were performed with aid of a ruler with scale in millimeters and results were expressed in centimeters. The assessment of DPAP was performed by the oven method, at 60 ºC, within which the seedlings were kept during a period of 72 h, and then were weighed in a centesimal precision analytical balance. With the results of LA and DPAP, the following parameters were determined: crop growth rate (CGR), expressed in $mg.day^{-1}$; relative growth rate (RGR), expressed in $mg^{-1} g^{-1}.day^{-1}$; and liquid assimilation rate (LAR), expressed in $mg.cm^{-2}.dia^{-1}$. These determinations were based on the methodology described in Gardner et al. (1985), in which : $CGR = (DM_2 – DM_1)/(T_2 – T_1)$; $RGR = (\ln DM_2 – \ln DM_1)/(T_2 – T_1)$; $LAR = (DM_2 – DM_1) * (\ln LA_2 – \ln LA_1)/(LA_2 – LA_1)$. Where: DM = dry mass; T = time; and LA = leaf area.

For assessment of physiological quality of seeds a completely randomized experimental design was used; and for assessment of initial growth of seedlings a randomized block experimental design was used, both of them with four replications. Data were subjected to ANOVA and analyzed by polynomial regression. For statistical analyses, the statistical analysis system Winstat , version 1.0, was used (Machado and Conceição, 2003).

Results and Discussion

By results achieved it is possible to verify that there has been no statistically significant effect for the parameters G and FCG, with the increase on the dosages of the Zn, B, and Mo micronutrients (Figures 1A and 1B). These data corroborate results found by Funguetto et al. (2010) that, in assessing seeds of rice have not observed statistically significant effect of Zn application on the seeds. Similar responses were obtained by Vieira and Moreira (2005) and Ohse et al. (2000). However, these results disagree from those results found by Ávila et al. (2006) that have observed significant increases on germination and vigor of corn (*Zea mays* L.) seeds treated with Zn, M, and B, in which the responses to application of these elements varied according to the corn hybrids assessed.

For the cropping of rice, Ohse et al. (2000) have also confirmed that treatment of seeds with Zn, B, and Cu can be applied to seeds prior to sowing without impairing their germination and vigor. Meireles et al. (2003) suggest that Mo improves seed physiological quality by capacity of this micronutrient in reorganizing the cellular membranes; thus avoiding losses of leachates during their imbibition process. Nevertheless, studies on coating of seeds with micronutrients have shown variability in results.

In relation to variable LAP (Figure 1C) it can be observed that the increase of dosages until 100 mL $.100 kg^{-1}$ seed of product used has led to an increase on aerial parts length of rice seedlings; however above such dosage a decrease on LAP has occurred. The values for RL and TLS have decreased with the increase on the dosages up to 200 $mL.100 kg^{-1}$ seed, agreeing with results found by Bays et al. (2007) that in a similar study have not observed statistically significant differences on length of seedlings with application of polymer and micronutrients based on Cobalt (Co), Mo, and B; although with addition of fungicide in the coating of seeds. These authors have described that the values for these parameters underwent only small variations; however, they have also observed that there has been difference on length of seedlings in relation to the dosages of the compound containing the micronutrients.

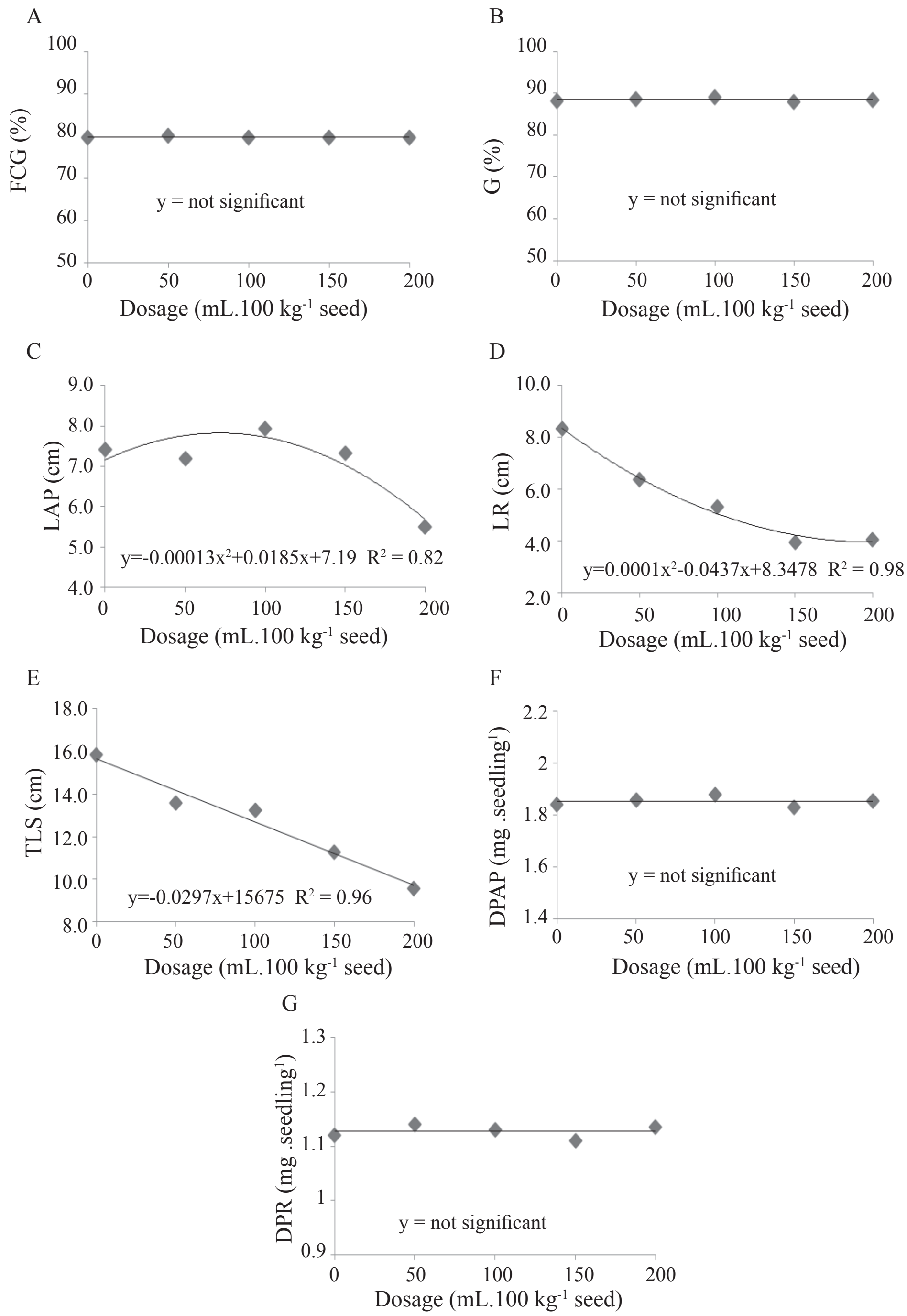

Figure 1. Polynomial regression for: germination (G) (A); first count of germination (FCG) (B); length of aerial parts (LAP) (C); length of roots (LR) (D); total length of seedlings (TLS) (E); dry phytomass of aerial parts (DPAP) (F); and dry phytomass of roots (DPR) (G), obtained from seedlings of lowland rice, cv. PUITA INTA-CL, originating from seeds treated with different dosages of a commercial product containing Zinc, Boron, and Molybdenum.

For the variables DPAP and DPR, the application of increasing dosages of micronutrients Zn, B, and Mo has not caused statistically significant effect (Figures 1F and 1G). These results are similar to results found by Bonnecarrère et al. (2004) for lowland rice, who have also concluded that there has been no statistically significant difference in relation to treatments with deficiency or sufficiency of Zn in the nutritive solution or in dosages applied, which did not differ between each other neither for DPAP nor DPR. However, Yagi et al. (2006) have detected a decrease on seed germination and in accumulation of dry mass of roots, as well as for the whole plant, in consequence of Zn application to seeds of sorghum (*Sorghum vulgare* Pers.). According to Oliveira et al. (2010), among the micronutrients by them assessed, B was the element that most reduced castor bean (*Ricinus communis* L.) seed germination, revealing rates lower than those presented by treatments that did not receive the micronutrient. Identical result has been found by Ohse et al. (2000), who have also observed reduction on vigor of rice seedlings by providing B via seeds. The authors have attributed such results to the high dosages of the nutrient applied to seeds; what may have caused phytotoxicity.

In regard to LA (Figure 2), it was detected a statistically significant increase for this variable in function of increasing dosages of the compound containing Zn, B, and Mo; being the volume of 200 mL .100 kg^{-1} seed the dosage that induced the best performance (Figure 2). As much in relation to dosages (Figure 2A) as in relation to assessment period (Figure 2B) assessed, it was observed a positive linear behavior for the variable LA.

On Figure 3 are shown data referring to DPAP of the rice plants treated with Zn, B, and Mo. The DPAP variable has generally presented quadratic behavior for all treatments assessed. It can also be observed that until 21 DAE, the values for DPAP have presented little increase along time. However, after that assessment period, the increase of these values was accentuated until 42 DAE. At that assessment period a 27% increase has occurred for DPAP in the dosage of 200 mL .100 kg^{-1} seed, in relation to treatment control. According to Ohse et al. (2000), Zn plays a role of growth promoter, and thus its application will be reflected on production of dry mass, similar to what has occurred in this experiment. Giordano and Mortvedt (1973) have also achieved increase of 9% on production of dry mass of rice plants when seeds were immersed in a zinc sulfate ($ZnSO^4$) aqueous solution, during 1 min., thus increasing the Zn absorption in 18%, in relation to control.

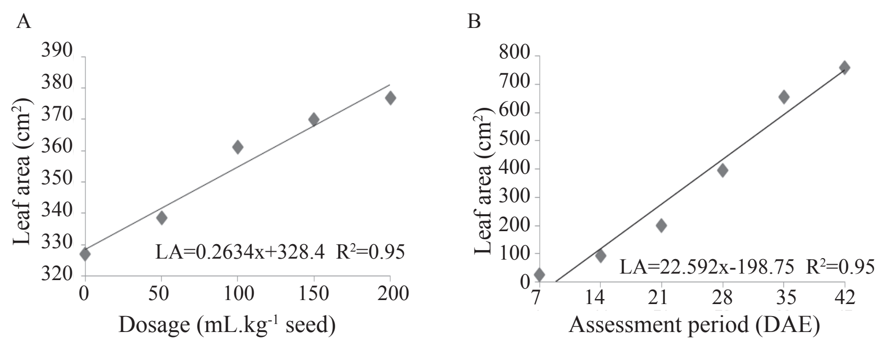

Figure 2. Polynomial regression for: leaf area (A) and assessment periods (B) of lowland rice seedlings, cv. PUITA INTA-CL, originating from seeds treated with different dosages of a commercial product containing Zinc, Boron, and Molybdenum. (DAE) = days after emergence.

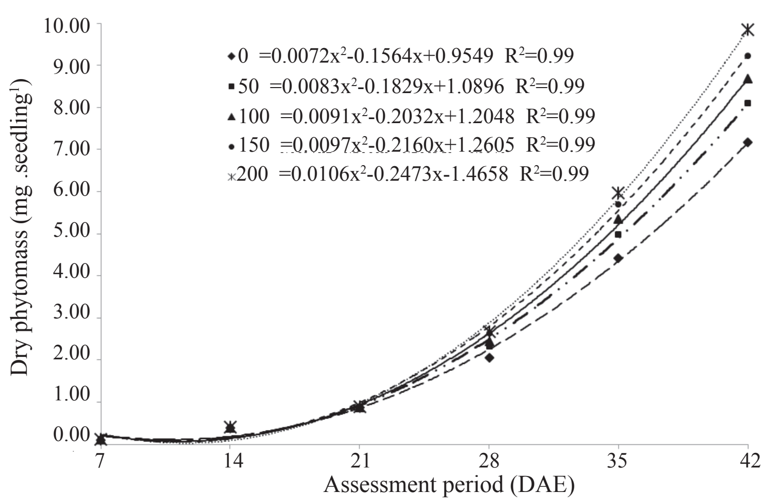

Figure 3. Polynomial regression for dry phytomass of aerial parts of lowland rice seedlings, cv. PUITA INTA-CL, originating from seeds subjected to treatments with different dosages of a commercial product containing Zinc, Boron, and Molybdenum. Regression analysis for dosages of product: 0 (control), 50, 100, 150, and 200 mL .100 kg^{-1} seed. (DAE) = days after emergence.

By data presented on Figure 4, it can be verified that height of rice plants, originating from seeds treated with Zn, B, and Mo has presented a linear, positive, and increasing behavior, showing such behavior as much in function of dosages (Figure 4A) as for assessment period (Figure 4B), being the dosage of 200 mL.100 kg^{-1} seed, the treatment that has presented the best performance, in relation to remaining treatments. Therefore, it is possible to infer that this result was due to the fact of Zn to be needed for tryptophan synthesis, an amino acid precursor of the indoleacetic acid (IAA), which is a hormone (auxin) promoter of growth in plants (Taiz and Zeiger, 2006); thus promoting an accentuated increases on plant height.

By observation of data presented on Table1, it is verifiable that plants subjected to treatments with Zn, B, and Mo did not undergo influence of dosages of product tested until assessment period of seven to 14 DAS for both the CGR and RGR. However, starting from that assessment period, occurred statistically significant increases with the increase of dosages of the product containing the microelements for the CGR variable. It can be also verified that variation of CGR explains the evolution of production of TDP and LA (Figures 2 and 3).

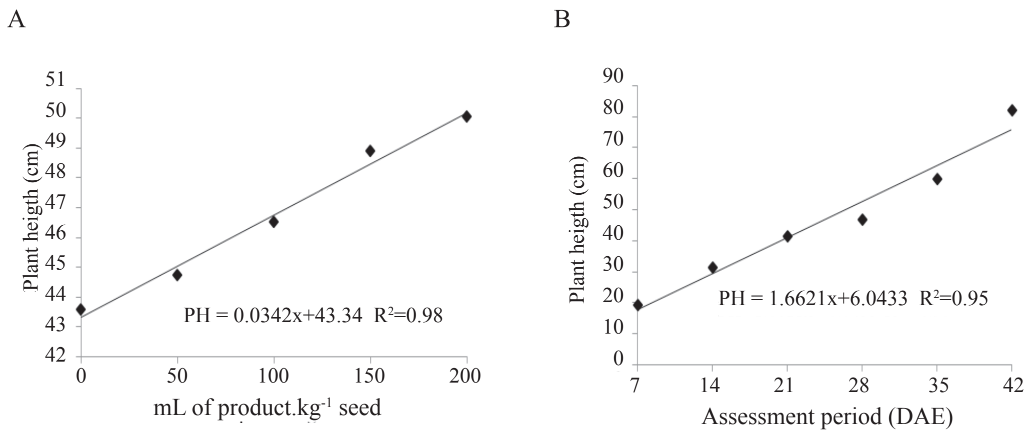

Figure 4. Polynomial regression for height of lowland rice plants, cv. PUITA INTA-CL, originating from seeds treated with different dosages of a commercial product containing Zinc. Boron, and Molybdenum (A) and assessed at different periods (B). (DAE) = days after emergence.

In regard to RGR, it can be observed that in the periods of assessment in what plants originating from seeds treated with the micronutrients have presented the highest productions of TDP and LA, the highest CGR has also occurred (Table 1). It has still been found that during the assessment period between 21 to 28 DAE the plants originating from seeds treated with Zn, B, and Mo have presented statistically significant differences among treatments for CGR only for the dosage of 200 mL.100 kg^{-1} seed; although without statistically significant differences among treatments for the remaining dosages (Table 1). In relation to LAR, differences statistically significant were not found among treatments, for all dosages and all assessment periods studied.

The treatment of lowland rice seeds with Zn, B, and Mo has little affected growth of plants; nevertheless, the vigor and germination of the seeds treated with these elements were not impaired by the treatment. The main effect of seed treatment with these micronutrients could be observed on yield. It is worthy to emphasize that within this study positive results were not obtained for germination; however, results statistically significant were obtained for LA, TDF (DFAP + DFR), and CGR until 42 DAE. Several researchers are obtaining positive results on germination and on the initial growth of seedlings through the application of Zn, B and Mo via seed treatment.

Table 1. Crop growth rate (CGR), relative growth rate (RGR), and liquid assimilation rate (LAR), obtained for lowland rice seedlings, cv. PUITA INTA-CL, originating from seeds treated with different dosages of a commercial product containing Zinc, Boron, and Molybdenum and assessed at different periods.

Dosage of product (mL.100 kg^{-1} seed)	Assessment period (Days after emergence)					
	0 to 7	7 to 14	14 to 21	21 to 28	28 to 35	35 to 42
CGR (mg.planta^{-1}.dia^{-1})						
0	15.17 ^{ns}	40.89 ^{ns}	69.19 ^{ns}	168.74 b*	338.11 b*	393.81 c*
50	15.61	40.68	69.11	174.02 ab	344.00 ab	400.67 bc
100	16.04	40.31	68.31	178.26 ab	346.75 ab	403.88 abc
150	16.21	40.91	69.30	182.05 ab	353.18 ab	411.37 ab
200	16.21	40.36	69.55	205.99 a	381.23 a	444.04 a
Mean	16.02	40.56	69.07	181.81	352.66	410.76
CV (%)	8.81	8.14	7.01	16.02	13.38	9.95
RGR (mg.g^{-1}.dia^{-1})						
0		5.30 ^{ns}	5.92 ^{ns}	6.65 b*	7.30 ^{ns}	7.67 ^{ns}
50		5.30	5.92	6.77 ab	7.32	7.69
100		5.30	5.92	6.82 ab	7.33	7.70
150		5.31	5.92	6.91 a	7.34	7.71
200		5.32	5.93	6.91 a	7.40	7.78
Mean		5.31	5.93	6.81	7.35	7.72
CV (%)		4.97	2.84	10.21	7.53	4.51
LAR (mg.cm^{2}.dia^{-1})						
0		0.760 ^{ns}	0.490 ^{ns}	0.620 ^{ns}	0.730 ^{ns}	0.620 ^{ns}
50		0.756	0.497	0.738	0.782	0.666
100		0.761	0.496	0.769	0.793	0.662
150		0.777	0.511	0.690	0.783	0.666
200		0.770	0.496	0.862	0.868	0.740
Mean		0.766	0.500	0.765	0.806	0.683
CV (%)		9.10	9.15	16.06	17.00	12.50

*Means followed by same letters for each response variable in the column do not statistically differ between each other by Tukey test, at 5% probability; ^{ns} = non significant.

Conclusions

The treatment of lowland rice with the commercial product Binova GRA®, containing Zn, B, and Mo, until the dosage of 200 mL.kg^{-1} seed, does not influence germination, first count of germination, production of dry phytomass of aerial parts, and dry phytomass of roots; nevertheless negatively influences the length of seedlings. In addition, promotes increase of leaf area, higher production of total dry phytomass and higher growth rate of seedlings, until 42 days after emergence.

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