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Biopriming in black oat seeds

Biopriming em sementes de aveia preta

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Abstract

This study performs the microbiolization of black oat seeds with *Trichoderma* spp. and *Bacillus subtilis* by physiological conditioning, suspension of biological structures, and film coating, improving the control of pathogens and the effect on seed germination and vigor. Microbiolization with suspension of biological structures was carried out with commercial products based on *Trichoderma* spp. and *Bacillus subtilis*. Water restriction was performed in PDA + Mannitol (-0.8 MPa) medium, for *Trichoderma* spp. and *B. subtilis*. One hundred disinfected seeds of black oat were distributed in each plate. Once root protrusion occurred in one seed, the others were removed and dried, under laboratory conditions, for 48h. Film coating was performed with the addition of polymer to the treatment syrup containing *Trichoderma* spp. or *B. subtilis*. The seeds were dried for 48 h in a laboratory environment. A treatment was used to coat the conditioned seeds with the organisms, individually or in combination. *Trichoderma* spp. and *B. subtilis* control the pathogens associated with black oat seeds. *B. subtilis* increases the germination, seedling performance and growth, and dry matter accumulation of black oat. *Trichoderma* spp. promotes seedling shoot growth and dry matter accumulation in black oat plants. However, *Trichoderma* spp. and *B. subtilis*, applied by physiological conditioning and film coating, compromise the germination and emergence of black oat seedlings.

Additional keywords: *Avena strigosa;* germination; physiological conditioning; polymer.

Resumo

O objetivo deste trabalho foi realizar a microbiolização de sementes de aveia-preta com *Trichoderma* spp. e *Bacillus subtilis* pelas técnicas de condicionamento fisiológico, suspensão de estruturas biológicas e peliculização, melhorando o controle de patógenos e o efeito sobre a germinação e vigor das sementes. A microbiolização com suspensão de estruturas biológicas foi realizada com produtos comerciais à base de *Trichoderma* spp. e *Bacillus subtilis*. A restrição hídrica foi realizada em meio BDA + Manitol (-0,8 MPa), para *Trichoderma* spp. e para *B. subtilis*. Em cada placa, foram distribuídas 100 sementes de aveia-preta desinfestadas. Ao ocorrer protrusão radicular na primeira semente, as demais foram retiradas e secas em ambiente de laboratório por 48 h. A peliculização foi realizada com a adição do polímero à calda de tratamento contendo *Trichoderma* spp. ou *B. subtilis*. As sementes foram secas por 48 h, em ambiente de laboratório. Foi utilizado um tratamento recobrindo as sementes condicionadas com os organismos, individualmente ou em associação. *Trichoderma* spp. e *B. subtilis* atuam no controle de patógenos associados às sementes de aveia-preta. *B. subtilis* promove a germinação de sementes de aveia-preta, o desempenho e o crescimento das plântulas, e o acúmulo de matéria seca em plantas. *Trichoderma* spp. proporciona crescimento de parte aérea de plântulas e acúmulo de matéria seca em plantas. Entretanto, *Trichoderma* spp. e *B. subtilis* aplicados por condicionamento fisiológico e peliculização comprometem a germinação e a emergência de plântulas de aveia-preta.

Palavras-chave adicionais: *Avena strigosa;* condicionamento fisiológico; germinação; polímero.

Introduction

Osmotic conditioning (water restriction, water conditioning, osmoconditioning or priming) consists of the slow and controlled hydration of seeds. It is used to increase germination uniformity and synchronization; seedling emergence and growth, even in low moisture soils; shoot growth rate; and to promote faster maturation (Diniz et al., 2006).

This technique has been used to improve seed yield in several species (Hölbig et al., 2011). It makes use of culture media for pathogen inoculation,

intensifying the infection without impairing seed germination. Working with inoculation of *Curtobacterium flaccumfaciens* pv, *flaccumfaciens* in bean seeds by means of physiological conditioning, Deuner et al. (2011) obtained efficient association of the bacteria with the seeds, without compromising physiological quality.

Another method for the treatment of seeds is the use of polymers that contribute to the distribution of products on the surface of seeds, giving good appearance and color uniformity (Avelar et al., 2015). Junges et al. (2013) used the association between film coating and water restriction and obtained better performance of the microbiolization of maize seeds with *Bacillus subtilis*. Diniz et al. (2006) used the technique of film coating to propagate *Trichoderma viride*, obtaining increased emergence and emergence rate in lettuce seedlings.

Seed treatment through the application of live microorganisms, such as *Trichoderma* spp. and *Bacillus subtilis*, has been used with satisfactory results, characterizing an alternative to the use of chemical inputs. This technique aims at the control of diseases and can be associated to other techniques with a view to promoting plant growth.

Trichoderma spp. is a biological control fungus that acts on several phytopathogens (Figueirêdo et al., 2010). It promotes plant growth and development by increasing nutrient availability, further raising yield and inducing disease resistance (Jegathambigai et al., 2010). Similarly, *Bacillus subtilis* acts directly or indirectly on the biocontrol of plant diseases (Leelasuphakul et al., 2008). According to the same authors, the direct antagonism exerted against phytopathogens occurs through antibiosis; synthesis of antimicrobial substances; parasitism; competition for space and nutrients; and synthesis of volatile compounds. The mechanism of indirect antagonism is induced by systemic resistance (Lanna Filho et al., 2010) through the synthesis of isoenzymes such as peroxidase and phenylalanine ammonium lyase (Martins et al., 2013).

The performance of *B.subtilis* in promoting plant growth has been reported for several species, being attributed to stimuli to the root system, with physiological increase of metabolites that trigger the sensitivity of the root system to external conditions, facilitating perception and nutrient absorption (Manjula & Podile, 2005).

In this context, this work aimed to control pathogens associated with black oat seeds, as well as to improve seed germination and vigor through microbiolization techniques using *Trichoderma* spp. and *Bacillus subtilis*. For that purpose, we used innovative techniques such as physiological conditioning, suspension of biological structures, and film coating, in isolation or in combination.

Material and methods

Certified seeds of black oat (*Avena strigosa* Schreb) cultivar Embrapa 139 (Neblina) were used. Before determining the proper water potential and applying the treatments, the seeds were disinfected with 1% sodium hypochlorite solution for 1 minute, followed by 70% alcohol for 1 minute and three baths in distilled and sterilized water.

The proper water potential for black oat seeds was determined as follows: physiological conditioning was performed in Petri dishes containing 50 mL of PDA medium plus solute mannitol at different water potentials (0.0; -0.6; -0.7; -0.8; and -0.9 MPa) (Coutinho et al., 2001). Then, 100 seeds were conditioned on the plates poured with the culture medium at different potentials. The plates were incubated in growth chambers (photoperiod of 12h at 25 °C) until root protrusion occurred in one seed. The remaining seeds were removed and dried in an air circulation chamber, at a temperature of 25 ºC for 48 h. Subsequently, the germination test was performed on germitest paper rolls.

Two hundred seeds were used for each potential tested, separated in eight replicates of 25. The germination test rolls were kept in a germinator, with 12h photoperiod and 25 ºC temperature, for five days, when the first germination count was performed. At 10 days, the percentages of germination, abnormal seedlings, and dead seeds were determined.

Biopriming was carried out by different microbiolization methods (water restriction, film coating, and suspension of biological structures), for two commercial biological products applied on black oat seeds: one based on a bacterial agent, *Bacillus subtilis*, and another based on a fungal agent, *Trichoderma* spp.

Nine microbiolization treatments were used as follows: T1: Physiological conditioning + *Trichoderma* spp.; T2: Film coating + *Trichoderma* spp.; T3: suspension of *Trichoderma* spp. spores; T4: Physiological conditioning + *Trichoderma* spp. followed by film coating; T5: Physiological conditioning + *Bacillus subtilis*; T6: Film coating + *Bacillus subtilis*; T7: suspension of *Bacillus subtilis* bacterial cells; T8: Physiological conditioning + *Bacillus subtilis* followed by film coating; T9: Physiological conditioning + *Trichoderma* spp. and *Bacillus subtilis* followed by film coating; T10: Chemical treatment (positive control); and T11: Untreated seeds (negative control).

Suspension of biological structures was applied as follows: Suspension of *Trichoderma* spp. spores was obtained from 10 g of the commercial product placed in 100 mL of distilled and sterilized water, applying 0.5 mL of the suspension to each 100 seeds; for *Bacilus subtillis*, 0.5 mL of the commercial product was applied to each 100 seeds. After microbiolization, the seeds were dried on filter paper, under laboratory conditions, for 48h.

Physiological conditioning: Performed in PDA culture medium plus solute mannitol (-0.8MPa), in which the organisms were replicated. *Trichoderma* spp. was isolated from the commercial product Agrotrich plus®; the plates were incubated for 10 days, so that the fungus sporulated abundantly. *Bacillus subtilis* was replicated with 0.5 mL of the commercial product Rhizoliptus® on each plate, being incubated for 48h for bacterial growth and development on the medium. In the joint microbiolization of the two organisms, 0.5 mL of the product based on *Bacillus subtilis* was applied to each 100 seeds. Subsequently, these seeds were conditioned on plates containing *Trichoderma* spp. One hundred previously disinfected black oat seeds were conditioned with the different organisms on each plate, being incubated in a germinator with photoperiod of 12h and temperature of 25 ºC, until root protrusion occurred in one seed. The remaining seeds were then removed from the medium and dried on filter paper, under laboratory conditions, for 48h.

Film coating: Red polymer (Color Seed® He) was applied according to the manufacturer's recommendation: 300 mL of the product for each 100 kg of seeds. A syrup was prepared for each 100 seeds by adding the amount of polymer relative to the weight of the sample, in 0.5 mL suspension of *Trichoderma* spp. or the commercial product based on *B. subtilis*. After, the seeds were dried on filter paper, under laboratory conditions, for 48 h.

Untreated seeds: The seeds were disinfected and dried under laboratory conditions for 48h.

Chemical treatment: Fungicide Captan SC (120 g a.i./100 kg seeds) was used diluted in 0.5 mL syrup of each treatment of 100 seeds.

To evaluate the performance of the seeds subjected to the different treatments, evaluations regarding health status, germination, seedling emergence, growth and development were carried out in plastic cups and under field conditions.

For measuring health status, 200 seeds per treatment were used, divided into eight replicates of 25 seeds. These were placed in gerboxes previously disinfected with 70% alcohol and 1% sodium hypochlorite, containing two sheets of sterile filter paper moistened with herbicide 2,4-D at 0.5% to inhibit germination. The seeds were incubated at 25 ºC with photoperiod of 12 h, for five days, being analyzed with the aid of a stereoscopic and optical microscope to observe the morphological structures of all the fungi present. The fungi were identified for genus, determining the percentage of incidence of each genus.

As described previously, the germination test was conducted with two counts, at five and ten days, according to methodology adapted from the Rules for Seed Analysis (Brasil, 2009). In the first count, we assessed the normal seedlings of each replicate, from which ten seedlings were randomly separated and measured for shoot and root length. Due to the reduced volume, the seedlings were grouped into four replicates of 20 seedlings for dry matter determination in an oven at 60 ºC for 48 h. At 10 days of incubation, seedlings were classified as strong normal, weak normal, and abnormal, also counting the number of dead seeds. Moreover, germination percentage was determined in each treatment, according to methodology adapted from the Rules for Seed Analysis (Brasil, 2009).

An experiment was carried out to evaluate the seedlings in a greenhouse, with daily irrigation. Four replicates of five seeds were sown in plastic cups containing 30 g of commercial substrate (Carolina Soil®). A completely randomized design was used, and the evaluation was performed 10 days after sowing. The following were evaluated: number of leaves, neck diameter, number of tillered plants, tiller length, root length, shoot length, dry root and shoot weight.

Emergence rate (ER) was determined in 200-cell alveolar trays, with one seed per cell, using the commercial substrate Carolina Soil®. Daily counts of emerged seedlings were carried out until a constant number was obtained. Thus, the emergence rate was determined by summing the number of plants emerged each day, divided by the number of days elapsed from sowing. After emergence became constant, the percentage of emerged seedlings was determined.

Field performance was evaluated in Santa Maria City, Rio Grande do Sul State. Sowing was done in a randomized complete block design with four replicates. In each plot, three rows spaced 20 cm apart were sown, and evaluations were performed at the center row. We used borders with untreated seeds between the plots, and 5 cm spacing between plants. Sowing was done manually, with two seeds per hole. Emergence percentage was evaluated at 18 days after sowing, followed by thinning, leaving only one plant per pot. At 28 days after sowing, seedling height was determined with a millimeter ruler, and the number of leaves per seedling was counted. When the plants showed 50% of flowering (80 days after sowing), the center row of each plot was collected. Plants were sectioned from the neck, and mean dry matter was determined in an oven at 60 ºC.

The mean of all variables was calculated and analysis of variance was performed using the F test at 5% probability. Means were compared using the Scott-Knott test at 5% probability, with the aid of SISVAR software (Ferreira, 2011).

Results and discussion

Root protrusion occurred in the first seed after 38 h of incubation for all potentials tested. The use of mannitol in the different potentials did not influence germination and first germination count (Table 1). However, the seedlings originated from treatments added with the restrictor produced a lower percentage of abnormal seedlings. The treatments did not differ in relation to the percentage of dead seeds. Although the potentials did not differ from each other in absolute values, the potential of -0.8 MPa provided the best performance of all variables evaluated.

Table 1 - Normal seedlings at four days (NS 4d), normal seedlings at ten days (NS 10d), dead seeds (DS) and abnormal seedlings (AS) of black oat seeds submitted to different water potential.

Treatments (MPa)	NS 4d (%)	NS 10d (%)	DS (%)	AS (%)
-0.0	28 a*	58 a	9 a	33 a
-0.6	59 a	74 a	18 a	8 b
-0.7	44 a	74 a	9 a	15 b
-0.8	83 a	92 a	2 a	6 b
-0.9	48 a	72 a	36 a	10 b
CV (%)	7	23	8	4

* Means followed by the same letter do not differ from each other by the Scott–Knott test at a 5% probability of error.

Initial seed germination was 88% and the first germination count was 73%. All microbiolization methods were similar for the efficiency of colonization by *Trichoderma* spp., where 100% of seeds were colonized (Table 2), showing good association with black oat seeds. The efficiency of pathogen control varied according to the microbiolization method, the organism used, and the pathogen genus. The use of physiological conditioning and film coating techniques enhanced the effect of *Trichoderma* spp., reducing the incidence of *Fusarium* sp. As for *B. subtilis*, suspension and coating of conditioned seeds provided the control of pathogens. Carvalho et al. (2011) obtained control of up to 51% of the incidence of *Fusarium oxysporum* f. sp. *phaseoli* in bean seeds by treatment with *Trichoderma harzianum*. For the incidence of *Penicillium* sp., only microbiolization suspension and film coating of seeds conditioned in the presence of *B. subtilis* did not control the fungus. All treatments significantly reduced the incidence of *Aspergillus* sp.

Table 2 - Incidence of *Trichoderma* spp. (TRI), *Fusarium* spp. (FUS), *Penicillium* spp. (PEN), *Aspergillus* spp. (ASP) and first count (FC), strong seedlings (SS), weak seedlings (WS), abnormal seedlings (AS) means in black oat seeds submitted to different treatments.

Treatments	TRI (%)	FUS (%)	PEN (%)	ASP (%)
Physiological conditioning + *Trichoderma* spp.	100 a*	0 b	1 c	0 d
Film coating + *Trichoderma* spp.	100 a	2 b	1 c	1 d
Suspension of *Trichoderma* spp. spores	100 a	7 a	0 c	0 d
Physiological conditioning + *Trichoderma* spp. followed by film coating	100 a	0 b	0 c	0 d
Physiological conditioning + *B. subtilis*	2 c	7 a	17 b	7 c
Film coating + *B. subtilis*	1 c	6 a	2 c	1 d
Suspension of bacterial cells of *B. subtilis*	7 b	0 b	24 a	21 b
Physiological conditioning + *B. subtilis* followed by film coating	0 c	0 b	29 a	6 c
Physiological conditioning + *Trichoderma* spp. and *Bacillus subtilis* followed by film coating	100 a	0 b	0 c	0 d
Chemical Treatment	0 c	6 a	0 c	0 d
No treatment	1 c	9 a	29 a	33 a
CV (%)	7	88	47	66
Treatments	FC (%)	SS (%)	WS (%)	AS (%)
Physiological conditioning + *Trichoderma* spp.	58 b*	61 c	7 a	15 a
Film coating + *Trichoderma* spp.	56 b	61 c	1 b	7 b
Suspension of *Trichoderma* spp. spores	73 b	76 b	3 b	8 b
Physiological conditioning + *Trichoderma* spp. followed by film coating	35 c	47 d	13 a	19 a
Physiological conditioning + *B. subtilis*	88 a	97 a	1 b	1 c
Film coating + *B. subtilis*	84 a	93 a	0 b	0 c
Suspension of bacterial cells of *B. subtilis*	69 b	90 a	2 b	0 c
Physiological conditioning + *B. subtilis* followed by film coating	85 a	95 a	3 b	0 c
Physiological conditioning + *Trichoderma* spp. and *Bacillus subtilis* followed by film coating	31 c	39 d	8 a	24 a
Chemical Treatment	66 b	82 b	8 a	3 b
No treatment	64 b	76 b	9 a	9 b
CV (%)	20	18	71	65

* Means followed by the same letter in the column do not differ from each other by the Scott–Knott test at a 5% probability of error.

These results confirm the action of *Trichoderma* spp. and *B. subtilis* in the control of pathogens associated with plant seeds and shoots. Similar results were observed by other authors when using seed microbiolization (Ludwig et al., 2009), where *Bacillus* spp. isolates were reported to have potential in the control of rice scald (*Gerlachia oryzae*). Furthermore, these isolates were also used in the control of fungi transmitted by white oat seeds (Silva et al., 2002). The application of *Bacillus subtilis* in the soil can also provide control of shoot diseases during the crop cycle, since it stands out as resistance inducer. In this sense, Araujo & Menezes (2009) verified the control of shoot diseases in tomato, with efficiency similar to the chemical treatment.

The treatments in which microbiolization with *B. subtilis* was carried out showed higher percentages of germination on paper roll (Figure 1) when compared to the control treatment or with *Trichoderma* spp. Similar results were obtained by Manjula & Podile (2005), which obtained a higher germination rate in pigeon bean seeds treated with a formulation based on *B. subtilis* AF1 in chitin-supplemented peat. However, the use of techniques such as physiological conditioning and film coating associated with seed microbiolization using *Trichoderma* spp. compromised seed germination. Using spore suspension as a microbiolization technique with the same organism, Carvalho et al. (2011) also found that there was no harm to the germination of healthy bean seeds.

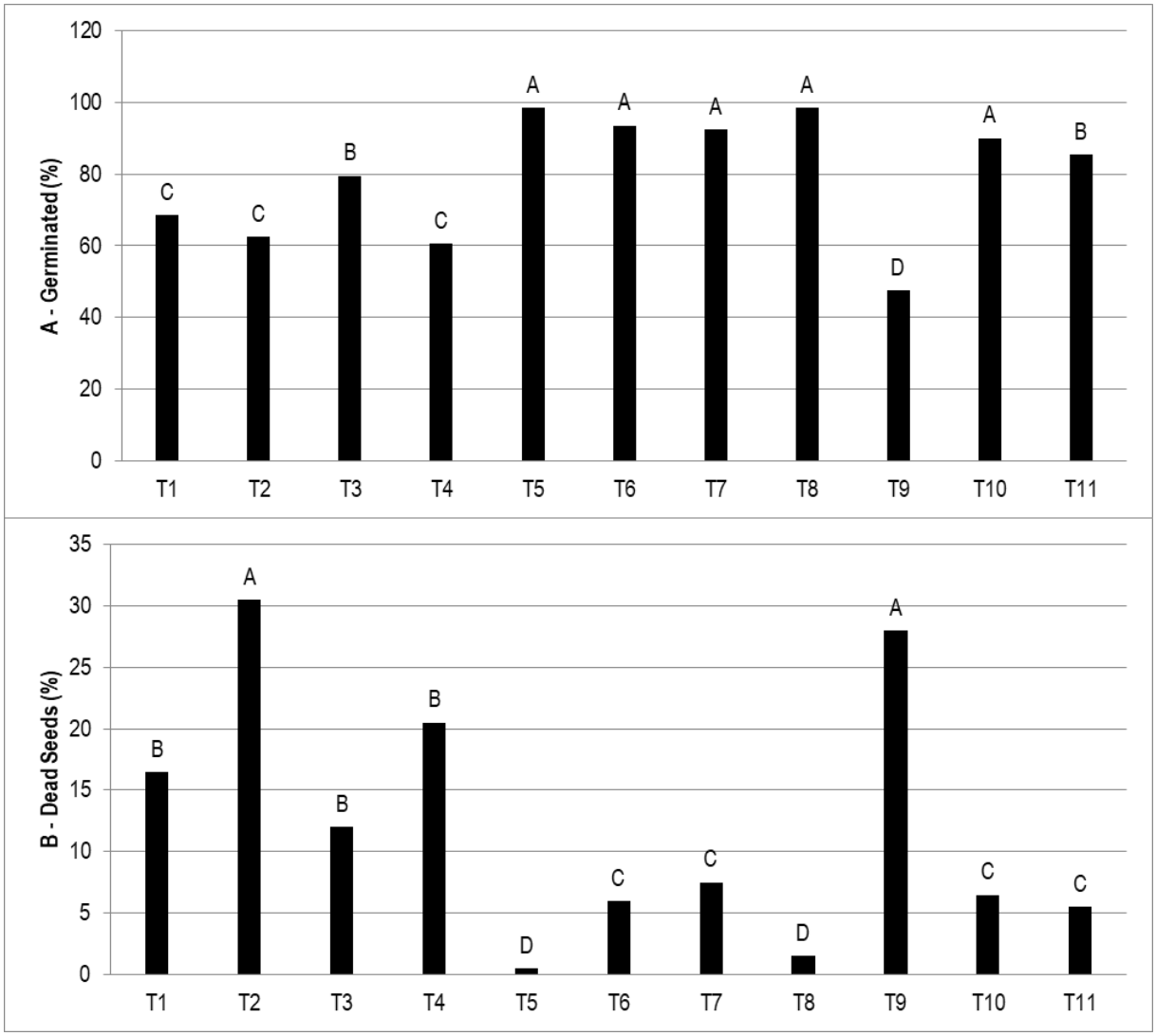

Figure 1 - Percentages of germinated (A) and dead seeds (B) measured in a germination test of black oat seeds submitted to various biopriming treatments (T1: Physiological conditioning + *Trichoderma* spp.; T2: Film coating + *Trichoderma* spp.; T3: Spore suspension of *Trichoderma* spp.; T4: Physiological conditioning + *Trichoderma* spp. followed by film coating; T5: Physiological conditioning + *Bacillus subtilis*; T6: Film coating + *Bacillus subtilis*; T7: Bacterial cell suspension of *Bacillus subtilis*; T8: Physiological conditioning + *Bacillus subtilis* followed by film coating; T9: Physiological conditioning + *Trichoderma* spp. + *Bacillus subtilis* followed by film coating; T10: Chemical treatment; T11: No treatment). Means followed by the same letter do not differ from each other by the Scott–Knott test at a 5% probability of error.

B. subtilis promoted a higher percentage of strong seedlings compared the other treatments, and the use of conditioning and film coating techniques allowed for faster seedling development in the first germination test (Table 2). Allied to this, the percentage of weak and abnormal seedlings was reduced. In contrast, *Trichoderma* spp. affected seedling performance, reducing the first germination count and the percentage of strong seedlings, and increasing the percentage of weak and abnormal seedlings; seed coating was the most detrimental treatment (T4 and T9).

Moreover, *B. subtilis* promoted greater root growth in all the methods used (Figure 2). Both *B. subtilis* and *Trichoderma* spp. promoted seedling shoot growth, however the response varied with the technique used. For *Trichoderma* spp., isolated conditioning showed the best performance, followed by seed coating, being still superior to untreated seeds. Likewise, Mwangi et al. (2011) emphasize that *T. harzianum* can be used to increase the growth of tomato seedlings. When evaluating seedling growth, it was observed that the suspension of *B. subtilis* bacterial cells provided lower results compared to the other techniques. The highest seedlings were obtained with seed conditioning, followed by seed coating. Dry matter accumulation was intensified by the adoption of other techniques, besides suspension, for microbiolization with *Trichoderma* spp., and by the convey related to *B. subtilis* coating.

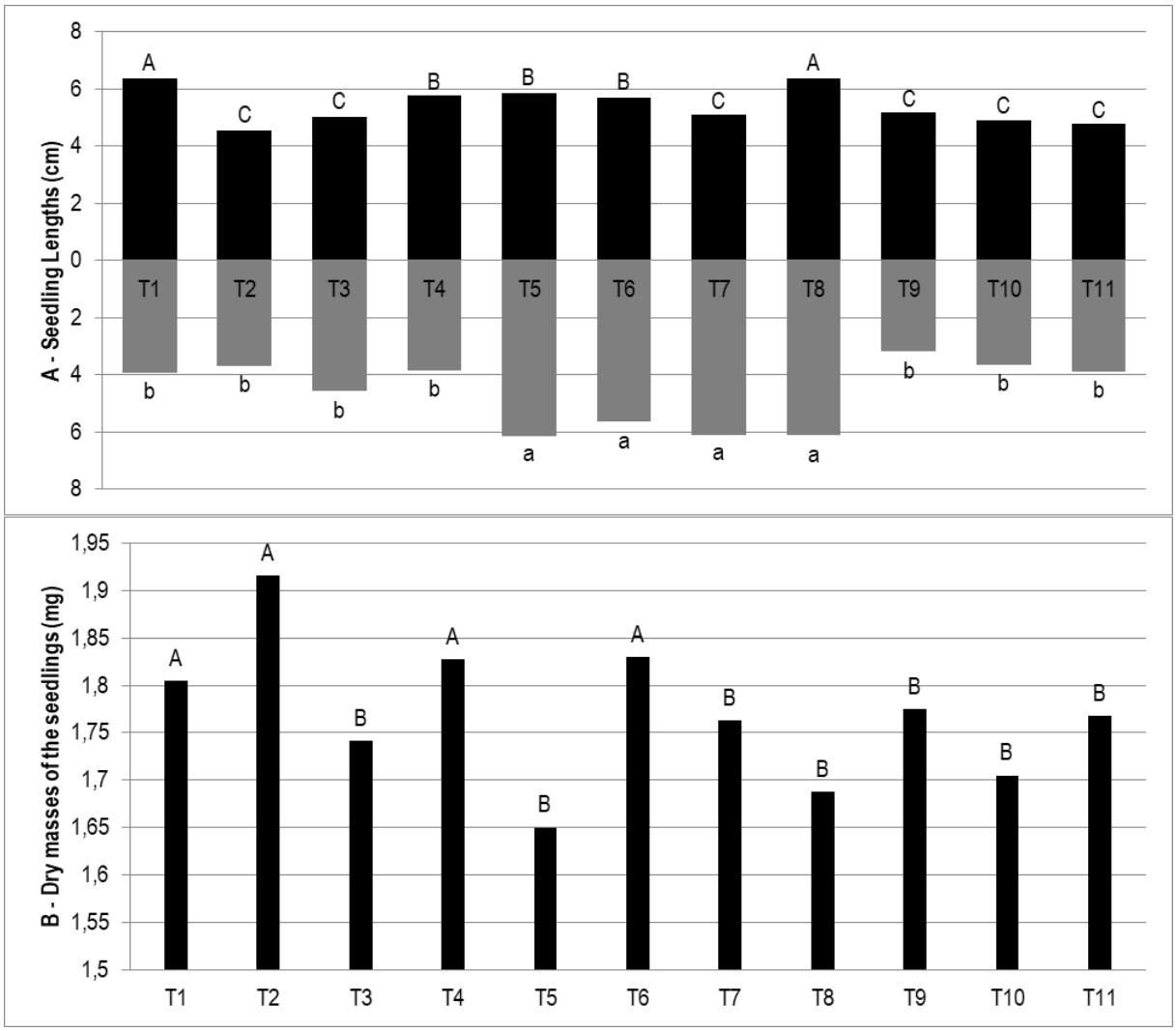

Figure 2 - Seedling root and shoot lengths (A) and dry mass of the seedlings (B) measured for germination tests of oat seeds submitted to various biopriming treatment (T1: Physiological conditioning + *Trichoderma* spp.; T2: Film coating + *Trichoderma* spp.; T3: Spore suspension of *Trichoderma* spp.; T4: Physiological conditioning + *Trichoderma* spp. followed by film coating; T5: Physiological conditioning + *Bacillus subtilis*; T6: Film coating + *Bacillus subtilis*; T7: Bacterial cell suspension of *Bacillus subtilis*; T8: Physiological conditioning + *Bacillus subtilis* followed by film coating; T9: Physiological conditioning + *Trichoderma* spp. + *Bacillus subtilis* followed by film coating; T10: Chemical treatment; T11: No treatment). Means followed by the same letter do not differ from each other by the Scott–Knott test at a 5% probability of error.

In the greenhouse experiment, under favorable conditions for seedlings, it was observed that among all the techniques used for microbiolization, the water restriction with *Trichoderma* reduced seedling emergence (Table 3) and seedling emergence rate. Faria et al. (2003) observed that cotton seeds treated with *T. harzianum* showed improvements in germination, emergence, and emergence rate. Neck diameter was favored by *Trichoderma* spp., conveyed by film coating, and by the association of the two organisms with the two techniques, with similar performance to the chemical treatment.

The microbiolization method used influenced the action of *Trichoderma* spp. on seedlings. For this organism, the method with suspension or the water restriction and film coating in isolation were responsible for a significant reduction in the average number of leaves, number of tillered seedlings, tiller length, shoot length, and shoot dry weight (Table 3). Other authors have observed deleterious effects of *Trichoderma* spp. on some crops: Carvalho et al. (2006) report the production of metabolites toxic to wheat coleoptiles by *T. viride*; Javaid & Ali (2011) observed herbicidal action of *T. harzianum*, *T. reesei*, and *T. pseudokoningii* metabolites on *Avena fatua*. Coating of conditioned seeds canceled this deleterious effect.

For *B. subtilis*, however, only the coating of seeds conditioned in the presence of the organism implied reductions in these variables. The effect of coating conditioned seeds contrasted between the two organisms. For *Trichoderma* spp., it was favorable, canceling out the deleterious effect produced in some variables; for *B. subtilis*, in turn, this was the treatment responsible for decreased performance. Hölbig et al. (2011) observed that the use of films in trials with coating of hydroconditioned onion seeds impaired seedling vigor. Junges et al. (2017) observed that the coating of forage turnip seeds, which had been osmotically conditioned in the presence of the same organisms, provided greater seedling shoot growth in the field. The root growth of black oat seedlings was only reduced with water restriction in isolation for the fungus, not representing a reduction in root dry matter, which did not differ between treatments.

Table 3 - Emergency (E), emergence speed index (ESI), stem diameter (SD), number of leaves (NL), number of seedlings with tillers (NST), tiller length (TL), shoot length of seedlings (SLS), root length (RL), shoot dry mass (SDM) measured in plantlets in greenhouse derived from black oat seeds under different treatments.

Treatments	E (%)	ESI	SD (mm)	NL	NST
Physiological conditioning + *Trichoderma* spp.	83.0 c*	4.4 b	1.8 b	2.3 b	2.2 b
Film coating + *Trichoderma* spp.	97.0 a	5.0 a	2.6 a	2.6 b	1.5 b
Suspension of *Trichoderma* spp. spores	99.0 a	5.1 a	1.8 b	2.4 b	1.2 b
Physiological conditioning + *Trichoderma* spp. followed by film coating	94.0 a	4.6 b	2.0 b	3.0 a	4.2 a
Physiological conditioning + *B. subtilis*	97.0 a	4.8 a	2.0 b	2.9 a	3.5 a
Film coating + *B. subtilis*	96.0 a	5.1 a	1.9 b	2.8 a	3.7 a
Suspension of bacterial cells of *B. subtilis*	98.0 a	4.9 a	2.1 b	2.9 a	4.7 a
Physiological conditioning + *B. subtilis* followed by film coating	98.0 a	4.8 a	1.7 b	2.5 b	2.7 b
Physiological conditioning + *Trichoderma* spp. and *Bacillus subtilis* followed by film coating	90.0 b	4.5 b	2.3 a	2.8 a	3.5 a
Chemical Treatment	100.0 a	4.7 b	2.6 a	2.7 a	3.7 a
No treatment	99.0 a	4.8 a	1.9 b	2.7 a	3.7 a
CV (%)	4.4	5.5	14.5	7.5	35.9

Treatments	TL (cm)	SLS (cm)	RL (cm)	MPA (mg)
Physiological conditioning + *Trichoderma* spp.	2.2 b*	9.5 b	12.1 b	78.7 b
Film coating + *Trichoderma* spp.	1.9 b	10.2 b	15.7 a	87.5 b
Suspension of *Trichoderma* spp. spores	1.1 b	9.4 b	15.3 a	73.0 b
Physiological conditioning + *Trichoderma* spp. followed by film coating	3.5 a	13.7 a	16.9 a	113.7 a
Physiological conditioning + *B. subtilis*	2.9 a	12.5 a	15.7 a	111.7 a
Film coating + *B. subtilis*	2.4 b	12.4 a	16.6 a	118.0 a
Suspension of bacterial cells of *B. subtilis*	3.7 a	14.4 a	17.9 a	156.5 a
Physiological conditioning + *B. subtilis* followed by film coating	1.8 b	9.9 b	16.4 a	89.5 b
Physiological conditioning + *Trichoderma* spp. and *Bacillus subtilis* followed by film coating	4.2 a	12.7 a	15.6 a	114.2 a
Chemical Treatment	2.7 a	12.1 a	18.5 a	114.2 a
No treatment	3.1 a	12.5 a	17.5 a	132.0 a
CV (%)	32.8	10.9	9.6	22.3

* Means followed by the same letter do not differ from each other by the Scott–Knott test at a 5% probability of error.

Under field conditions, there was a significant effect of treatments, in some of which emergence was impaired (Figure 3). Physiological conditioning and film coating for *Trichoderma* spp., and suspension of bacterial cells for *B. subtilis*, as well as chemical treatment, decreased seedling emergence. There was no significant effect on seedling height and number of leaves. Notwithstanding, the dry matter accumulation in adult plants, at flowering, was favored by physiological conditioning and film coating associated with *Trichoderma* spp., and by physiological conditioning and suspension of *B. subtilis* cells, as well as by chemical treatment. Seed conditioning and seed coating in isolation were superior to both the associated use and the spore suspension of *Trichoderma* spp. For *B. subtilis*, microbiolization associated with physiological conditioning or suspension had superior performance. This increased dry matter accumulation represents an important contribution to crop yield.

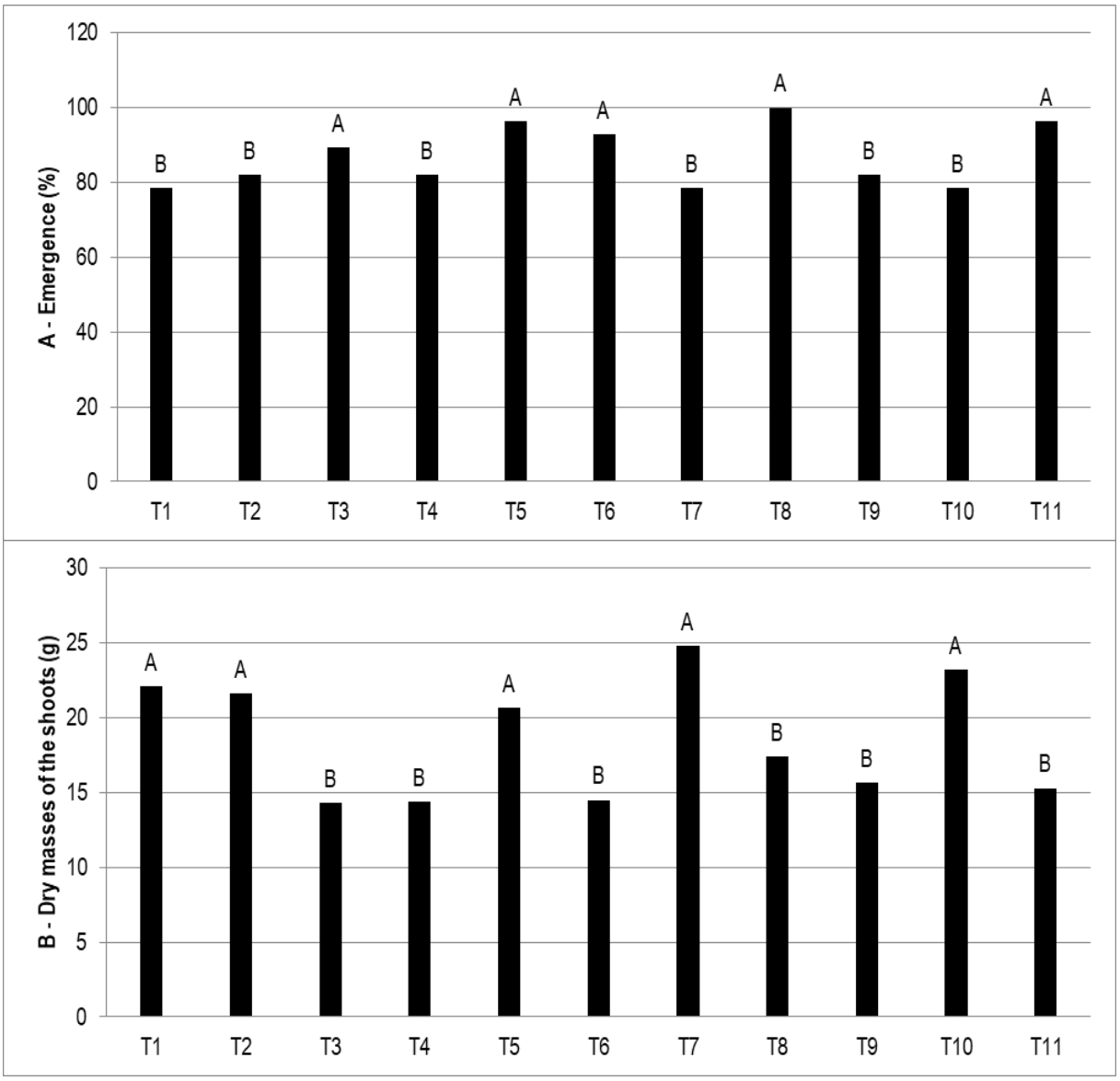

Figure 3 - Emergence (A) and shoot dry mass (B) in adult plants from black oat seeds submitted to various biopriming treatments (T1: Physiological conditioning + *Trichoderma* spp.; T2: Film coating + *Trichoderma* spp.; T3: Spore suspension of *Trichoderma* spp.; T4: Physiological conditioning + *Trichoderma* spp. followed by film coating; T5: Physiological conditioning + *Bacillus subtilis*; T6: Film coating + *Bacillus subtilis*; T7: Bacterial cell suspension of *Bacillus subtilis*; T8: Physiological conditioning + *Bacillus subtilis* followed by film coating; T9: Physiological conditioning + *Trichoderma* spp. + *Bacillus subtilis* followed by film coating; T10: Chemical treatment; T11: No treatment). Means followed by the same letter do not differ from each other by the Scott–Knott test at a 5% probability of error.

Conclusions

For grain yield, plant height, and number of rows per ear, the performance of hybrids BRS 1010 and DKB 390 VT PRO2 coincide, regardless of the plant population.

The maize crop developed better in the sowings performed on Jan 20 and Feb 07, and late sowings negatively affect yield.

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