Volumen 41, Nº 2. Páginas 63-70
IDESIA (Chile) Junio, 2023

Interference of resistance inducers on the physiological and sanitary quality of beet root seeds and seedlings

Interferencia de inductores de resistencia en la calidad fisiológica y sanitaria de semillas y plántulas de remolacha

Letícia Camara Vieira[1], Vanessa Neumann Silva[1], Paola Mendes Milanesi[2], Julia Andrade[2]*

ABSTRACT

High physiological and sanitary quality seeds and seedlings, resulting in a uniform and pathogen-free plant, require further study of possible approaches to improve seed potential, tolerance, and resilience, including the use of resistance inducers. This study aims to determine how different resistance inducers affect the physiological performance and sanitary quality of seeds and seedlings of beetroot (*Beta vulgaris L.)*. The treatment of beet seeds was carried out with a control group (distilled water), *Bacillus amyloliquefaciens*, and *Trichoderma harzianum*. Germination, seedling length, dry mass, seed health, protein quantification in plant tissues, and phenylalanine ammonia-lyase activity were all assessed. The resistance inducers had no adverse effects on the physiological potential of seeds treated with all evaluated inducers. Regarding the sanitary quality of seeds, beet seed treatments with *Bacillus amyloliquefaciens* doses of 3.0 and 6.0 mL L^{-1} of and *Trichoderma harzianum* doses equal to or greater than 0.125 mL L^{-1} significantly reduced the incidence of *Fusarium* sp. Only the *Trichoderma harzianum* treatment reduced the incidence of pre-emergence *Damping off* at all doses tested. A significant effect was observed in reducing total protein contents in seeds treated with *Bacillus amyloliquefaciens* and *Trichoderma harzianum*.

Keywords: *Trichoderma harzianum*, *Bacillus amyloliquefaciens*, *Beta vulgaris* L.

RESUMEN

*Para garantizar semillas y plántulas de alta calidad fisiológica y sanitaria, que den como resultado una planta uniforme y libre de patógenos, se deben investigar medidas que puedan mejorar el potencial, la tolerancia y la resiliencia de la semilla, como el uso de inductores de resistencia. El objetivo de este estudio es determinar cómo diferentes inductores de resistencia afectan el comportamiento fisiológico y la calidad sanitaria de semillas y plántulas de remolacha (*Beta vulgaris *L.). El tratamiento de semillas de remolacha se realizó con un testigo (agua destilada),* Bacillus amyloliquefaciens *y* Trichoderma harzianum*. Se evaluaron la germinación, la longitud de las plántulas, la masa seca de las plántulas, la salud de las semillas, la cuantificación de proteínas en los tejidos de las plantas y la actividad de la fenilalanina amoniaco-liasa. Los inductores de resistencia no tuvieron efectos negativos sobre el potencial fisiológico de las semillas tratadas con todos los inductores evaluados. En cuanto a la calidad sanitaria de las semillas, los tratamientos de semillas de remolacha con dosis de* Bacillus amyloliquefaciens *de 3,0 y 6,0 mL L^{-1} y de* Trichoderma harzianum *iguales o superiores a 0,125 mL L^{-1} redujeron significativamente la incidencia de* Fusarium *sp. Solo el tratamiento con* Trichoderma harzianum *redujo la incidencia de* Damping off *previo a la emergencia, en todas las dosis probadas. Se observó un efecto significativo en la reducción del contenido de proteína total en semillas tratadas con* Bacillus amyloliquefaciens *y* Trichoderma harzianum*.*

Palabras clave*:* Trichoderma harzianum, Bacillus amyloliquefaciens, Beta vulgaris *L.*

Introduction

Beetroot is a vegetable of the Amaranthaceae family that is considered a high-nutritional-value food and one of the most nutritious plant species, being a source of pigment compounds that play significant roles in human nutrition and metabolism.

Beets are commercially propagated through seeds, and numerous biotic and abiotic factors influence their production. Seed quality affects

1 Universidade Federal da Fronteira Sul, campus Chapecó. Chapecó, Santa Catarina, Brasil.
2 Universidade Federal da Fronteira Sul, campus Erechim, Rio Grande do Sul, Brasil.
* Autor para correspondência: vanessa.neumann@uffs.edu.br

Fecha de Recepción: 26 de octubre, 2022.
Fecha de Aceptación: 24 de enero, 2023.

productivity, and the formation of a suitable plant stand; for beets, root production, and technological value are the consequence of intricate interactions between the cultivar's genetic potential, seed quality, production site conditions, and technologies applied (Michalska-Klimczak *et al*., 2018). Furthermore, low-vigor seeds are more sensitive to the effects of phytopathogenic fungi, lowering their physiological performance and yield (Soares *et al*., 2016).

Seeds with sanitary quality are those that are free of phytopathogens. Fungi are the primary agents responsible for production losses and seed quality decrease. Seed-borne pathogens are responsible for diminished seed germination capacity and for variance in plant morphology in the field, which can reduce crop yields (Gebeyaw, 2020).

Large amounts of pesticides are used to achieve high sanitary quality, creating environmental and consumer health interference (Carvalho, 2012). As a result, applying alternative controls provides a possibility for mitigating such effects.

An alternative that has shown to be promising is the use of induced resistance, defined by the use of inducing agents that promote an increase in plant resistance levels without modifying its genome (Carvalho, 2012). Several compounds, including *Bacillus amyloliquefaciens* (Gowtham *et al*., 2018) and Trichoderma (Ferrigo *et al*., 2020), among others, have been demonstrated to be effective in creating resistance to pathogens in several cultivated species.

The treatment of seeds with resistance inducers can be a cost-effective way to obtain seeds with high physiological and sanitary quality, resulting in the formation of a stand of healthy and vigorous plants; however, there are few results in the literature on the use of this technique for beet seeds at the moment. Therefore, this study aims to determine how different resistance inducers affect the physiological performance and sanitary quality of beet seeds and seedlings.

Material and methods

Laboratory and field tests were conducted in three stages: Step 1: Effect of resistance inducers on beet seed physiological potential; Step 2: Effect of resistance inducers on beet seed sanitary quality; Step 3: Effect of resistance inducers on beet seedling health. A completely randomized design was employed in steps 1 and 2, and a randomized block design was used in step 3. The treatments used for all stages were control (distilled water), *Bacillus amyloliquefaciens*, and *Trichoderma harzianum* at different concentrations.

Seed treatment

Beetroot seeds of the Vermelha Comprida cultivar without any chemical treatment were used. The treatments used were: a) Control: only distilled water; b) *Bacillus amyloliquefaciens*: the commercial product NemaControl® (SIBI BS 10 isolate (CCT 7600) 5 x 10^9 CFU/mL; 30 g L^{-1}) at concentrations of 1.5; 3.0 and 6.0 mL L^{-1}, following the manufacturer's recommendations; c) *Trichoderma harzianum*: the product Trichodermil (strain ESALQ-1306; minimum of 2.0 x 10^9 viable conidia mL^{-1}; 48 g L^{-1}) at concentrations of 0.125, 0.25 and 0.50 mL L^{-1}, following the manufacturer's recommendations.

For the resistance inducers *Bacillus amyloliquefaciens* and *Trichoderma harzianum*, the mixture prepared with the treatments was distributed over the seeds in a Petri dish, which was then left to dry naturally on a bench for 24 hours.

After carrying out the treatments, the tests described below were performed for this stage: Germination test: the methodology adapted from that described in the Rules for Seed Analysis (Brazil, 2009a) was used, with five repetitions of 50 seeds for each treatment distributed in plastic boxes of the gerbox type (11.5 x 11.5 x 3.5 cm), on germitest paper substrate, being previously moistened with distilled water in a volume 2,5 times the weight of dry paper, and which were kept in a germination chamber at a temperature of 20 ºC. During the germination test, the evaluations were carried out according to the criteria established by the Rules for Seed Analysis at four and 14 days after sowing (DAS), counting normal and abnormal seedlings and dead seeds. Seedling growth and dry mass: 20 seedlings were evaluated per experimental unit, from which the length of roots and shoots was determined using a ruler graduated in millimeters. Twenty seedlings evaluated for length were placed in paper bags and dried in an oven at 65 ºC for 72 hours before being weighed on a precision balance (0.0001 g) to determine the dry mass. Seed health (Blotter test method with freezing): 20 replications with 20 seeds were used for a total of 400 seeds per treatment. The seeds were placed on two sheets of germitest paper inside a plastic gerbox box and incubated for 5 days at 20 ºC with a photoperiod of 12 hours (Brasil, 2009b). A stereomicroscope was used to analyze the seeds individually to identify pathogenic fungi. The results were expressed as a percentage of fungal incidence to two decimal places.

Assessment of the effect of seed treatment with resistance inducers on the sanitary quality of seedlings.

The beet seeds were first covered with the resistance inducers' doses before being placed in plastic trays to develop vegetable seedlings with 162 cells each, and Mecplant® vegetable substrate was used for this purpose. Sowing was performed manually in the growing trays, with one seed per cell. The experimental unit contained 50 cells per tray, replicating each treatment four times. The trays were kept in a shade netting structure, and irrigation and cleaning procedures were performed. The assessments were conducted 14 days after sowing, following a methodology adapted from Mazaro (2009), to determine the incidence of damping-off after emergence (% of fallen seedlings) and measurement of the length of the aerial part of seedlings with a millimeter ruler.

For the biochemical analysis, samples of 20 beet seedlings were used, which were taken at the end of the field experiment from each treatment and repetition. Immediately after collection, the samples were stored in a freezer until phenylalanine ammonia lyase (PAL) activity and total proteins were analyzed. This stage was completed in the Phytopathology Laboratory. Protein quantification in plant tissues: The proteins in the samples were determined using Bradford's Coomassie Blue method (1976), with bovine serum albumin as a standard. The measurements were taken using a 595nm spectrophotometer and expressed in mg g^{-1} of tissue. The activity of phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) was determined using the approach reported by *Rodrigues et al.* (2006). The enzymatic extract was prepared by macerating 1.0 g of plant material in 6 mL of TRIS-HCL buffer (pH 8.0); the difference in absorbance caused by the conversion of phenylalanine to trans-cinnamic acid was predictive of PAL activity. The samples were analyzed in a 290 nm spectrophotometer, and the results were expressed in mg g^{-1} of tissue.

The data obtained in all experiments were subjected to analysis of variance and regression test ($p < 0.05$), separately for each inducer, using the SISVAR statistical software.

Results and discussion

There were no symptoms of post-emergence damping-off in beet seedlings for any of the evaluated inducers or in the control treatment, and the presence of disease-specific symptoms such as anasarca in the stem - at the height of the seedling neck, constrictions, or seedlings felled during the experiment were not observed.

In terms of the blotter test, overall, there was no evidence of *Rhizoctonia solani* mycelium associated with the seeds for any studied inducers or the control treatment. However, pre-emergence damping-off symptoms, such as the formation of anasarca spots and radicle, which are related to the structures of many fungi, were detected in all treatments tested.

Damping-off control is hampered by the presence of various pathogens, including *Rhizoctonia, Pythium, Phytophthora*, and *Fusarium* fungi, which can act alone or in combination (Lamichhane *et al.*, 2017). As a result, it was not possible to specify the disease's causal agent using the blotter test method, only the occurrence of symptoms in beet seedlings; therefore, the results were expressed as a percentage of seedlings with Damping off.

However, it is worth noting the presence of the genus *Fusarium* sp. in all treatments tested, with the presence of white mycelium with a dense cottony appearance. *Fusarium equiseti* has been identified as an etiological agent of seedling damage in beet in the United States of America (Haque; Parvin, 2020).

In general, when beet seeds were treated with *Bacillus amyloliquefaciens*, increases in the variable of germination % were seen when compared to the control treatment, with superior performance at the dose of 1.5 mL L^{-1} (Figure 1). The germination percentage of tomato seedlings treated with *B. amyloliquefaciens* rose by 14.37% compared to the control in another study (Sultana *et al.*, 2020). These findings could be linked to increased production of phytohormones including, cytokinin, auxins, and gibberellins (Diaz *et al.*, 2021).

The doses of *Bacillus amyloliquefaciens* used did not affect the variables shoot length, root length, and seedling dry weight, nor the percentage of emergence seedlings and shoot length of emerged seedlings (Table 1). The phytohormones released can stimulate growth at specific concentration levels but may be ineffective at lower concentrations (Biswas *et al.*, 2000).

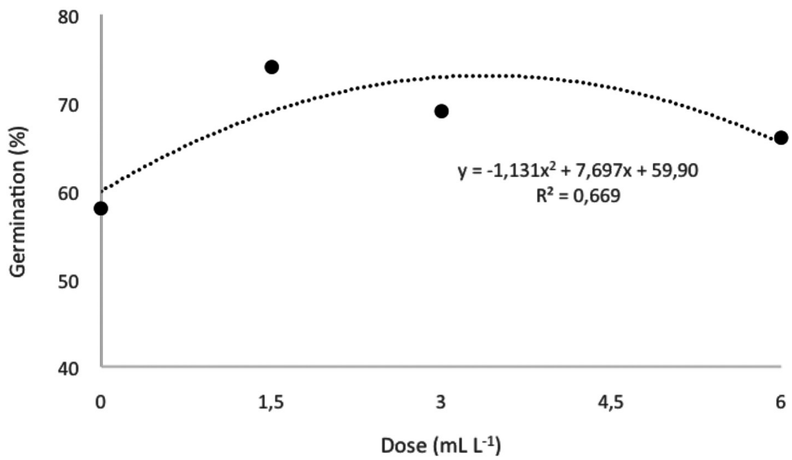

Figure 1. Germination of beet seeds treated with *Bacillus amyloliquefaciens.*

Table 1. Mean values of seedling dry mass (SDM), shoot length (SL) and roots length (RL), seedling emergence (SE), shoot length of emerged seedlings (SLES), *Fusarium incidence* (Fi), PAL activity (PAL) and seedlings with Damping off (DOS) in beetroot seeds as a function of the treatment with different doses of *Bacillus amyloliquefaciens*.

Variables	Doses (mL L^{-1})			
	0	1.5	3.0	6.0
SDM (g/seedling)	1.4^{ns}	1.3	1.4	1.4
SL (cm)	2.30^{ns}	2.53	2.42	2.50
RL (cm)	4.06^{ns}	4.38	4.26	4.56
SE (%)	88^{ns}	86	87	83
SLES (cm)	5.95^{ns}	5.94	5.31	5.76
FI (%)	11.5c*	0.75b	0.0a	0.0a
FAL (Uabs/min/mg of protein)	0.05^{ns}	0.05	0.06	0.06
DOS (%)	1.95^{ns}	2.0	1.85	1.95

* Means followed by the same letter on the line do not differ by Tukey's test ($p < 0.05$).
NS: not significant, in the analysis of variance ($p < 0.05$).

There was also no significant effect for phenylalanine ammonia-lyase quantification or the percentage of seedlings with pre-emergence Damping-off symptoms (Table 1). Treatment with *B. amyloliquefaciens* considerably reduced the incidence of Damping off induced by *Sclerotium rolfsii* in tomatoes (Sultana *et al.*, 2020). These results reveal that its antagonist effect varies between cultures, causal agents, and even rhizobacteria isolates, as seen in cucumber, where only two isolates out of 37 were able to attenuate the symptoms of pre-emergent damping-off (Lucon *et al.*, 2008).

In an experiment to verify *Bacillus cereus* resistance in tomato seeds, no changes in phenylalanine ammonia-lyase activity were identified (Silva *et al.*, 2004). PAL is an important enzyme to indicate plant stress; its activity can be regulated by biotic and abiotic factors such as light, temperature, herbivory, mechanical damage, growth regulators, and fungal and viral diseases, among others (Castro *et al.*, 2020). In this case, it may not have been induced due to the low percentage of pathogens and stresses. Another hypothesis would be that the PAL enzyme had reduced its levels by the end of the experiment.

Significant results were reported for the incidence of *Fusarium* sp. (Table 1), with doses of ≥ 3 mL L^{-1} producing the best outcomes. Studies report that genes involved in the biosynthesis of antifungal agents are found in *Bacillus amyloliquefaciens*, being capable of inhibiting spore germination and suppressing the growth of *Fusarium oxysporum* (Zhao *et al.*, 2013).

Regarding protein quantification, there was only a minor decrease in the results evaluated when the concentration of *Bacillus amyloliquefaciens* compared to the control (Figure 2). One explanation for the decrease in protein levels is that the proteins generated are membrane proteins, which are challenging to work with due to their insolubility in aqueous solutions and, in many cases, poor stability in detergent micelles (Carlson *et al.*, 2018).

Regarding the results of the *Trichoderma harzianum* treatment of beet seeds, no variations in the physiological potential of beet seeds were observed for germination, shoot and root length, and seedling dry mass variables (Table 2).

This effect can be considered favorable because the treatment with *T. harzianum* is capable of harming seedling development, as demonstrated in the tomato crop, resulting in a decrease in the growth of the seedlings produced (Romagna *et al.*, 2019). The unfavorable effect generated by Trichoderma isolates may be due to the synthesis of compounds that hinder seedling development when present in concentrations larger than the optimal range (Vinale *et al.*, 2008). Thus, up to a concentration of 0.50 g/L of *Trichoderma harzianum*, no adverse effects on beet seedling growth and development are found.

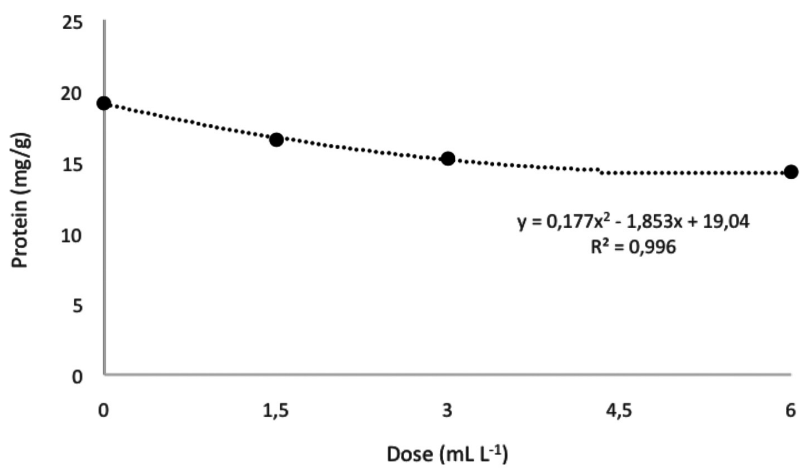

Figure 2. Mean of protein quantification values in beet seedlings as a function of seed treatment with different doses of *Bacillus amyloliquefaciens*.

Table 2. Mean values of germination (G), seedlings length (SL) and root length (RL), seedling dry mass (SDM), seedling emergence (SE), shoot length of emerged seedlings (SLES), and seedlings with Damping off (DOS) from beet seeds treated with different doses of *Trichoderma harzianum*.

Variables	Doses (mL L^{-1})			
	0	0.125	0.25	0.50
G (%)	55^{ns}	68	59	64
SL (cm)	3.25^{ns}	3.60	3.08	3.23
RL (cm)	3.83^{ns}	3.65	3.39	3.48
SDM (g/seedling)	0.92^{ns}	0.92	0.96	0.98
SE (%)	92^{ns}	89	91	92
SLES (cm)	6.13^{ns}	5.09	6.09	6.07
DOS (%)	1.75b*	0.0a	0.0a	0.0a
FAL (Uabs/min/mg of protein)	0.0047^{ns}	0.0055	0.0052	0.0055

* Means followed by the same letter on the line do not differ by Tukey's test ($p < 0.05$).
NS: not significant, in the analysis of variance ($p < 0.05$).

T. harzianum doses did not have significantly affect the percentage of emerging seedlings or shoot length of emerged seedlings (Table 2). Given that there were no negative impacts on the physiological potential or development of beet seedlings, the treatment with *Trichoderma harzianum* is a viable resistance inducer.

The regression analysis revealed significant findings for the percentage of seedlings with pre-emergence Damping off. The percentage of seedlings with pre-emergence Damping off in beet seeds using the blotter test decreased with a dose of 0.125 mL L^{-1} of *T. harzianum*, demonstrating its potential in controlling fungal diseases (Table 2). Elshahawy and El-Mohamedy (2019) found similar results for tomato seedlings; in a greenhouse experiment, the combined inoculation of Trichoderma isolates (*Trichoderma harzianum, T. asperellum, T. virens*) suppressed damping-off induced by *Pythium aphanidermatum* and increased tomato plant survival by 74.5%.

In terms of *Fusarium spp.* control, the doses of the resistance inducer *T. harzianum* had a considerable effect, with a rapid decline in the percentage of pathogen occurrence (Figure 3A). The authors observed signs of lysis and disintegration in the attacked species in a study evaluating the effectiveness of *T. harzianum* in inhibiting mycotoxin-producing fungi, which occurred due to the excretion of enzymes involved in lysis, such as chitinases, capable of reducing the growth of Aspergillus. flavus, *Aspergillus carbonarius, Alternaria alternata,* and *Fusarium oxysporum* (Braun *et al.*, 2018).

A growth in the activity of Phenylalanine ammonia-lyase was observed with the increase in the doses used, correlating the concentration of more significant PAL activity with a lower incidence of *Fusarium* spp., demonstrating that the treatment of beet seeds with *Trichoderma harzianum* can promote biochemical changes in response to seedling defense (Figure 3B). The

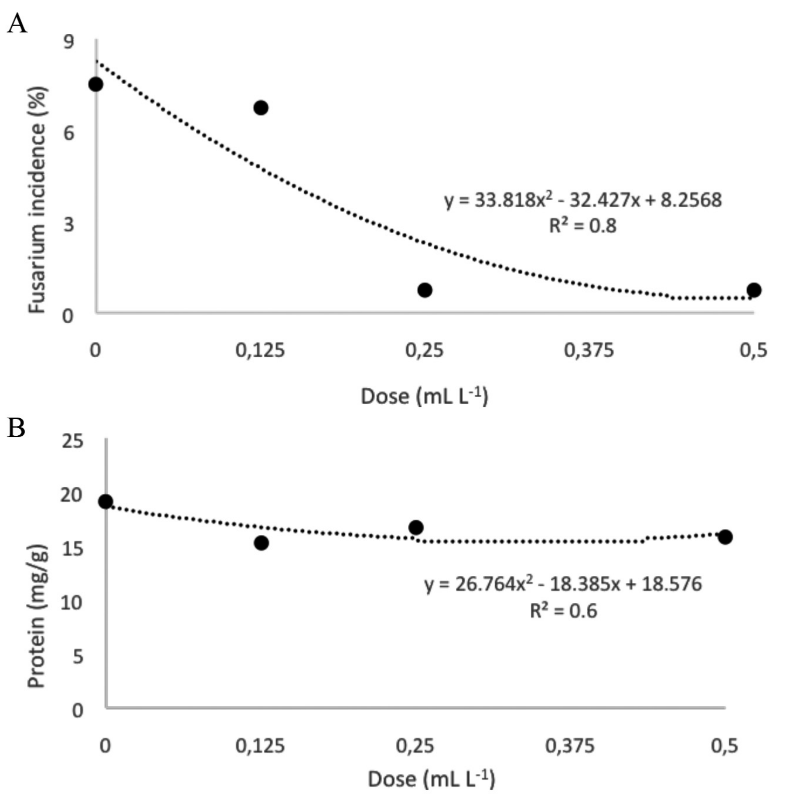

Figure 3. Mean Incidence Values of *Fusarium* sp. (A) and quantification of proteins (B) in beet seedlings, as a function of seed treatment with different doses of *Trichoderma harzianum.*

activity of phenylalanine ammonia-lyase determines the ability of pathogenic fungi to induce systemic acquired resistance (Duba *et al.*, 2019). PAL is a crucial enzyme for lignin and phytoalexins, strengthening the host cell wall against pathogen penetration (Naguib *et al.*, 2021).

Regarding protein quantification, the measured values dropped as the concentration of T. *harzianum* rose, as was observed with *Bacillus amyloliquefaciens* (Table 2). Similarly, the synthesized proteins may have been insoluble in aqueous buffers, or their quantities may have been lowered towards the experiment's conclusion when quantification was conducted (Voet and Voet, 2013).

Conclusion

There were no harmful impacts on the physiological potential of beet seeds generated by any investigated inducers. Regarding seed sanitary quality, beet seed treatments with *Bacillus amyloloquefaciens* doses of 3.0 and 6.0 mL L^{-1} and *Trichoderma harzianum* doses equal to or greater than 0.125 mL L^{-1} effectively reduced the incidence of *Fusarium* sp. The incidence of pre-emergence Damping off in beet seedlings was reduced only with treatment with *Trichoderma harzianum* at all doses tested. There was a significant effect on the reduction of total protein levels in seeds treated with *Bacillus amyloloquefaciens* and *Trichoderma harzianum*.

Literature cited

Biswas, J.C.; Ladja, J.K.; Dazzo, F.B. Yanni, Y.G.; Rolfe, B.G. 2000. Rhizobial inoculation influences seedling vigor and yield of rice. *Agronomy Journal*, 92(5): 880-886.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2): 248-254.

Brasil. Ministério da Agricultura e Reforma Agrária. 2009a. Regras para análise de sementes. Brasilia, Brasil, 2009a. 395 p.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento 2009b. Manual de Análise Sanitária de Sementes. Brasilia, Brasil. 200 p.

Braun, H.; Woitsch, L.; Hetzer, B.; Geisen, R.; Zange, B.; Schmidt-Heydt, M. 2018. Trichoderma harzianum: inhibition of mycotoxin producing fungi and toxin biosynthesis. *International Journal of Food Microbiology*, 280(2): 10-16.

Carlson, M.L.; Young, J.W.; Zhao, Z.; Fabre, L.; Jun, D.; LI, J.; Dhupar, H.S.; Wason, I.; Mills, A.T.; Beatty, J.T.; Klassen, J.S.; Rouiller, I.; Duong, F. 2018. The Peptidisc, a simple method for stabilizing membrane proteins in detergent-free solution. *eLife,* 7: e34085.

Carvalho, N.L. 2012. Resistência genética induzida em plantas cultivadas. *Electronic Journal of Management, Education and Environmental Technology*, 7(7): 1379-1390.

Castro, A.H.F.; Coimbra, M.C.; Fonseca, S.T.D.; Souza, A.A.M. Phenylalanine ammonia-lyase in higher plants: a key enzyme for plant development. In: In: Taylor, J.C. (Ed.). Advances in Chemistry Research. Nova Science Publishers: New York, USA. pp. 83-120.

Diaz, P.A.E; Gil, O.J.A.; Barbosa, C.H.; Desoignies, N.; Rigobelo, E.C. 2021. *Aspergillus* spp. and *Bacillus* spp. as Growth Promoters in Cotton Plants Under Greenhouse Conditions. *Frontiers in Sustainable Food Systems*, 5: e709267.

Duba, A.; Goriewba-Duba, K.; Wachowska, U.; Glowacka, K.; Wiwart, M. 2019. The Associations between Leaf Morphology, Phenylalanine Ammonia Lyase Activity, Reactive Oxygen Species, and Fusarium Resistance in Selected Species of Wheat with Different Ploidy Levels. *Plants*, 8(10): e360.

Elshahawy, I.E.; El-Mohamedy, R.S. 2019. Biological control of Pythium damping-off and root-rot diseases of tomato using Trichoderma isolates employed alone or in combination. *Journal of Plant Pathology*, 101: 597-608.

Ferrigo, D.; Mondin, M.; Ladurner, E.; Fiorentini, F.; Causin, R.; Raiola, A. 2020. Effect of seed biopriming with Trichoderma harzianum strain INAT11 on Fusarium ear rot and Gibberella ear rot diseases. *Biological Control*, 147: e104286.

Gebeyaw, M. 2020. Review on: Impact of Seed-Borne Pathogens on Seed Quality. *American Journal of Plant Biology,* 5(4): 77-81.

Gowtham, H.G.; Murali, M.; Singh, S.B.; Lakshmeesha, T.A.; Murthy, K.N., Amruthesh, K.N., Niranjana, S.R. 2018. Plant growth promoting rhizobacteria-Bacillus amyloliquefaciens improves plant growth and induces resistance in chilli against anthracnose disease. *Biological Control*, 126: 209-217.

Haque, M.E.; Parvin, M.S. 2020. First Report of Fusarium equiseti Causing Leaf Yellowing and Stunting on Sugar Beet (Beta vulgaris L.) in Montana, USA. *Journal of Plant Physiology & Pathology*, 8 (4): 1.

Lamicchane, J.R.; Durr, C.; Schwanck, A.A.; Robin, M.H.; Sarthou, J.P.; Cellier, V.; Messean, A.; Aubertot, J.N. 2017. Integrated management of damping-off diseases, a review. *Agronomy for Sustainable Development*, 37: e10.

Lucon, C.M.M.; Akamatsu, M.A.; Harakava, R. 2008. Promoção de crescimento e controle de tombamento de plântulas de pepino por rizobactérias. *Pesquisa Agropecuária Brasileira*, 43(6): 691-697.

Mazaro, S.M.; Wagner Júnior, A.; Dos Santos, I.; Citadin, I., Possenti, J.C.; De Gouvêa, A.
2009. Controle do tombamento de plântulas de beterraba e tomate pelo tratamento de sementes com quitosana. *Pesquisa Agropecuária Brasileira*, 44 (11): 1424-1430.

Michalska-Klimczak, B.; Wyszyński, Z.; Pačuta, V.; Rašovský, M.; Różańska, A.
2018. The effect of seed priming on field emergence and root yield of sugar beet. *Plant Soil Environment*, 64(5): 227-232.

Naguib, D.M.; Alzandi, A.A.; Shamk, I.M.; El-Houda; Reyad, N.E.H.A.
2021. Fabatin induce defense-related enzymes in cucumber against soil born pathogen, Fusarium oxysporum. *Rhizosphere*, 19: e100381.

Rodrigues, A.A.C.; Bezerra Neto, E.; Coelho, R.S.B.
2006. Indução de resistência a Fusarium oxysporum f. sp. tracheiphilum em caupi: eficiência de indutores abióticos e atividade enzimática elicitada. *Fitopatologia Brasileira*, 31(5): 492-499.

Romagna, I.S.; Junges, E.; Karsburg, P.; Pinto, S.Q.
2019. Bioestimulantes em sementes de olerícolas submetidos a testes de germinação e vigor. *Scientia Plena*, 15(10): 2-6.

Silva, H.S.A.; Romeiro, R.S.; Macagnan, D.; Halfeld-Vieira, B.A.; Pereira, M.C.B.; Mounteer, A.
2004. Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. *Biological Control*, 29(2): 288-298.

Soares, V.N.; Rodrigues, H.C.S.; Gadotti, G.I.; Meneghello, G.E.; Villela, F.A.
2016. Influence of fungi associated with watermelon seeds on physiological and health quality. *Australian Journal of Crop Science*, 10(6): 852-856.

Sultana, S.; Paul, S.C.; Parveen, S.; Alam, S.; Rahman, N.; Jannat, B.
2020. Isolation and identification of salt-tolerant plant-growth-promoting rhizobacteria and their application for rice cultivation under salt stress. *Canadian Journal of Microbiology*, 66: 144-160.

Vinale, F.; Sivasithamparam, K.; Ghisalbertic, E.L.; Marra, R.; Barbetti, M.J.; LI, H.; Woo, S.L.; Lorito, M.
2008. A novel role for Trichoderma secondary metabolites in the interactions with plants. *Physiological and Molecular Plant Pathology*, 72(1-3): 80-86.

Voet, D.; Voet, J.G.
2013. *Bioquímica*. 4. ed. São Paulo: Artmed, 1504 p.

Zhao, P.; Quan, C.; Wang, Y.; Wang, J.; Fan, S.
2013. Bacillus amyloliquefaciens Q-426 as a potential biocontrol agent against Fusarium oxysporum f. sp. spinaciae. *Journal of Basic Microbiology*, 54(5): 448-456, 2013.