(DOI): 10.5935/PAeT.V8.N1.05

Brazilian Journal of Applied Technology for Agricultural Science, Guarapuava-PR, v.8, n.1, p.47-54, 2015

Cientific Paper

Salicylic acid on the induction of resistance of tomato plants and control of *Rhizoctonia solani* Kuhn *in vitro*

Douglas Junior Bertoncelli[1]
Rita de Cacia Dosciatti Serrão Rocha[1]
Sergio Miguel Mazaro[2]
Jean Carlo Possenti[2]
Maristela dos Santos Rey Borin[2]

Abstract

Among the diseases that affect the tomato culture, a special attention is given to the seedling damping off due to the damages it causes on the initial phase of the cultivation. We aimed in this study to assess the different concentrations of salicylic acid (SA) on the induction of resistance to the damping off of tomato seedlings and the control of *Rhizoctonia solani* Kuhn *in vitro*. The seed treatment of tomato plants was conducted with immersion in SA solutions for 5 minutes under the concentrations of 0.5; 1.0; 1.5 and 2.0 mMe and control (distilled water). Subsequently, we showed them in expanded polystyrene trays containing the substrate Plantmax Florestal®, previously sterilized and inoculated with R.solani, being the experimental unit constituted by 20 cells, in 4 replicates. The experiment was conducted over 14 days in cultivation chamber with control of temperature (23oC ±2), luminosity (photoperiod of 12 hours) and humidity (70% ±10); then we assessed the seed germination, incidence of damping off, seedling length and fresh matter mass. We also quantified in the tissues of the plants the levels of the enzymes phenylalanine ammonia-lyase (FAL), β-1,3-glucanase and chitinase. In the experiment in vitro, the experimental unit was constituted by a Petri dish, in four replicates, being the SA incorporated to the BDA (potato dextrose agar) and assessed the mycelial growth of R.*solani*. The AS application in the tomato plant seeds do not interfere on the incidence of seedling damping off, but it induces resistance, activating the enzyme β-1,3-glucanase. The SA did not present effect over the R.*solani, in vitro.*

Keywords: *Solanum lycopersicum* Mill, elicitor, resistance inducer, PR protein.

Ácido salicílico en la inducción de resistencia a *Rhizoctonia solani* Kuhn en tomate

Resumen

Entre las enfermedades que afectan a la cultura de tomate se tiene atención especial a "damping off" de plántula debido a los daños que causa en la fase inicial del cultivo. El objetivo de este estudio fue evaluar las diferentes concentraciones de ácido salicílico (AS) en la inducción de resistencia a la fusariosis de plántulas de tomate y el control de *Rhizoctonia solani* Kuhn *in vitro.* El tratamiento de las semillas de las plantas de tomate se llevó a cabo con la inmersión en soluciones durante 5 minutos bajo las concentraciones AS de 0,5; 1,0; 1,5 y 2,0 mMe y el control (agua destilada). Posteriormente, fueran sembrados en bandejas de poliestireno expandido con el sustrato Plantmax Florestal®, esterilizado y inoculado con *R. solani*, siendo la unidad experimental constituido por 20 celdas, en 4 repeticiones. El experimento se llevó a cabo durante 14 días en cámara de cultivo con control de la temperatura (23° C±2), luminosidad (fotoperíodo de 12 horas) y humedad (70 ± 10%); en seguida se evaluó de la germinación de las semillas, la incidencia de damping off, la

Received at: 31/08/14 Accepted for publication at: 20/01/15

1 Programa de Pós- Graduação em Agronomia. Universidade Tecnológica Federal do Paraná – UTFPR. Câmpus Pato Branco. Via do Conhecimento, s/n - Fraron, Pato Branco – PR. Email: dj_bertoncelli@hotmail.com; ritadserrao@hotmail.com;

2 Dr. Prof. Universidade Tecnológica Federal do Paraná – UTFPR. Câmpus Dois Vizinhos. Estr. p/ Boa Esperança, S/n - Zona Rural, Dois Vizinhos– PR. Email: sergio@utfpr.edu.br; jpossenti@utfpr.edu.br; maristelarey@utfpr.edu.br

longitud de las plántulas y la masa de materia fresca. También se cuantificó en los tejidos de las plantas los niveles de las enzimas fenilalanina amonio-lyase (FAL), beta-1,3-glucanasa y quitinasa. En el experimento in vitro, la unidad experimental estuvo constituida por la placa de Petri, en cuatro repeticiones, siendo el AS incorporado a la PDA (agar dextrosa de patata) se evaluó el crecimiento micelial de *R. solani*. La aplicación de AS en las semillas de tomate no interfiere en la incidencia de damping off, pero induce resistencia, con la activación de la enzima β-1,3-glucanasa. El AS no presentó efectos sobre *R. solani, in vitro*.

Palabras clave: *Solanum lycopersicum* Mill, elicitor, inductor de resistencia, PR proteínas.

Ácido salicílico na indução de resistência em tomate e *Rhizoctonia solani* Kuhn

Resumo

Dentre as doenças que afetam a cultura do tomateiro, atenção especial é dada ao tombamento de plântulas devido aos danos que causa na fase inicial da cultura. Objetivou-se neste trabalho avaliar diferentes concentrações de acido salicilico (AS) na indução de resistência ao tombamento de plântulas de tomateiro e no controle de *Rhizoctonia solani* Kuhn *in vitro*. O tratamento das sementes de tomateiro foi realizado, com imersão em solução de AS por 5 min nas concentrações de 0,5; 1,0; 1,5 e 2,0 mM e a testemunha (água destilada). Em seguida foram semeadas em bandejas de poliestireno expandido contendo o substrato Plantmax Florestal® previamente esterilizado e inoculado com R. solani, sendo a unidade experimental constituída por 20 células, em 4 repetições. O experimento foi conduzido por 14 dias em câmara de cultivo com controle de temperatura (23oC ±2), luminosidade (fotoperíodo de 12horas) e umidade (70% ±10) e então avaliada a germinação das sementes, incidência de tombamento, comprimento de plântula e massa da matéria fresca. Foi também quantificado nos tecidos das plântulas os teores das enzimas fenilalanina amônia-liase (FAL), β-1,3-glucanase e quitinases. No experimento in vitro, a unidade experimental foi constituída por uma placa de Petri, em 4 repetições, sendo o AS incorporado ao meio BDA (Batata-dextrose e Agar) e avaliado o crescimento micelial de *R.solani.* A aplicação de AS nas sementes de tomateiro não interfere na incidência de tombamento de plântulas, mas induz a resistência, ativando a enzima β-1,3-glucanase. O AS não apresentou efeito sobre *R. solani, in vitro.*

Palavras chave: *Solanum lycopersicum* Mill, elicitor, indutor resistência, proteína-RP

Introduction

The tomato plant (*Solanum lycopersicum* Mill.) is original from the South America, being later transported to Europe by the Spanish in the sixteenth century, when it was initially cultivated as an ornamental plant in the gardens of Spain, Italy and England, from where it spread to other regions of the world (ALVARENGA, 2004).

The world production of tomato in 2010 was of 151.69 million tons. China was the largest tomato producer worldwide in 2010, with 31% of the world production. The Brazilian production was of 4.11 million tons and was the 9th largest, corresponding to about 3% of the world total (SEAB, 2012).

The tomato cultivation presents susceptibility to various types of diseases during its life cycle; although a special attention is given to the damping-off, because it is considered one of the major problems of plant diseases, due to the fact that its control or prevention is hampered by the involvement of several pathogens that act in isolation or combined, characterizing a disease that attacks culture at the seedling phase, being able to occur on the pre- and post-emergence (STEPHENS et al., 1981).

Among the fungi complex that cause damping-off, the *Rhizoctonia solani* is considered the most harmful, for causing, in higher intensity than the others, the damping-off on the pre-emergence, besides the one at post-emergence (GRANDO, 2002).

Among the control methods of this disease, the alternative controls have been more and more studied, mainly by the use of inducers or elicitors that activate the plant resistance to pathogens (MAZARO et al, 2009).

It is considered as a concept of resistance induction the activation of a vegetal defense state against diseases, being induced systemically in plants by the use of external biotic or abiotic agents, without any alteration of the plant's genome, being called elicitors (STADNIK, 2000; HAMMERSCHIMIDT et al., 2001).

The elicitor salicylic acid (AS) acts as an indicator in plants, especially on the defense against pathogens, being transported via the phloem to the non-infected parts of the plant, mainly in the form of methyl salicylate (MARTINEZ et al., 2000). The AS regulates the resistance mechanisms in the infection location through the systemic acquired resistance (RSA), which results on the synthesis of the phytoalexins and the PR proteins (HEIL; BOSTOCK, 2002).

KATARIA et al. (1997), observed that the salicylic acid and the chloro-salicylic acid inhibited 100% of the mycelial growth of *Rhizoctonia solani* in the concentration of 10 mM, while the acibenzolar-S-methyl reduced in 50% in the concentration of 2 mM.

Due to the potential of resistance induction of the salicylic acid (AS), and to the lack of studies on the control of seedling damping-off of tomato plants, through the seed treatment, this study aimed to assess different concentrations of AS on the resistance induction to the damping-off of tomato seedlings and the control of *Rhizoctonia solani, in vitro*.

Material and Methods

The experiments were carried out at the Federal Technological University of Paraná – Dois Vizinhos Campus, and the cultivation stage conducted in a controlled chamber (phytotron system), installed in the Vegetal Physiology Laboratory and in vitro in the phytopathology laboratory of the same institution, in the year of 2014.

The seed treatment was conducted with immersion in AS solutions for 5 minutes under the concentrations of 0.5; 1.0; 1.5 e 2.0 mM and the control (distilled water), in an entirely random design with four replicates. Following, we sowed them in expanded polystyrene trays containing the substrate Plantmax Florestal®, previously sterilized and inoculated with *R.solani*. Each repetition was constituted by 20 cells, where each cell received a seed treated with the inducer.

The *R.solani* mycelium was previously inoculated in autoclaved wheat seeds, and maintained in a B.O.D. incubator. These wheat seeds contaminated by R.solani were used as a contaminant mean to the sterilized substrate, at the proportion of 10g kg^{-1}. The inoculums were incorporated to the substrate three days before receiving the seeds.

The trays were maintained in the cultivation chamber with dimensions of 2.5 m length x 2.5 m width x 2.5 m height, with controlled temperature (23oC ±2), luminosity (photoperiod of 12 hours) and humidity (70% ±10). After 14 days, we finalized the experiment, analyzing the variables of seed germination, damping-off incidence, seedling length and fresh matter mass. In the control treatments and at the highest concentration of AS (2.0 mM), we also quantified in the tissues of the plants the levels of the enzymes phenylalanine ammonia-lyase (FAL), β-1,3-glucanase and chitinase, whereas the samples were constituted by 0.5 g, mixed in all parts of the vegetal (leaves, stem, root), which were frozen and stored immediately after in liquid nitrogen until the evaluations.

The emergence percentage was assessed considering the number of germinated seedlings, and the percentage was determined considering the total percentage of 20 seeds per replicate. The percentage of damping-off was considered the number of plants that presented symptoms of the disease. The size of the seedlings was determined with the aid of a caliper rule. The production of total fresh matter mass (plant root and shoot) of the seedlings was assessed considering the weight of fresh mass; before the weighing, the roots were washed and the mass determined in a precision scale.

The determination of the activity of the phenylalanine ammonia-lyase (FAL) was conducted by colorimetric quantification of the trans-cinnamic acid released from the substrate phenylalanine, according to the methodology described by Kuhn (2007), where he used 0.25 g of the sample with more 3.0 mL of the buffer TRIS – HCl pH 8.0. This extract was conditioned in eppendorf tubes and centrifuged for 10 minutes at 4°C and 6,000 rpm. After, we transferred an aliquot of 200 µL for test tubes, adding more 3.0 mL of extraction buffer. The solution was stirred in vortex, obtaining, thus, the enzymatic extract. From this extract, 1.5 mL was transferred to

another test tube, with more 1.0 mL of the extraction buffer and 0.5 mL of phenylalanine. Again, this solution was stirred in vortex for homogenization. And after, the tubes were incubated in Bain Marie for 45 minutes at 40°C. After being removed from the Bain Marie, the tubes were placed in ice bath for 5 minutes to interrupt the reaction and thus be able to conduct the reading in spectrophotometer at 290 nm.

For the dosage of the activities of chitinase and β-1,3-glucanase, the samples were macerated into 2.0 mL of buffer acetate 10 mM (pH 5.0), with posterior centrifugation (20,000 g per 25 minutes at -4°C). The supernatant was collected and used for assessing the activity of the enzymes. The enzymatic activity of the chitinase was assessed through the release of soluble fragments of "CM-chitin-RBV", from carboxymethylated chitin marked with bright violet remazol. For the spectrophotometric determination of the activities of β-1.3-glucanase in the extracts, we used as substrate a solution of bright blue carboxymethyl curdlan-remazol (CM-Curdlan-RBB 4 mg ml^{-1}, Loewe Biochemica GmbH), according to the methodology developed by WIRTH e WOLF (1992) and to the procedure described by GUZZO et al. (1996).

The experiment *in vitro* was conducted at the Phytopathology Laboratory of the UTFPR, Dois Vizinhos Campus, being used the concentrations of 0.5; 1.0; 1.5 e 2 mM of AS, besides a control treatment containing distilled water, The experimental design was of entirely randomized with four replicates.

The inducer was incorporated to the cultivation mean B.D.A, with electromagnetic stirrer for homogenizing the mixture. After, the cultivation mean was place on a Petri® plate, in a laminar flow hood. After the solidification of the mean, we placed disks with 10 mm diameter, containing the mycelium *R.solani* on the plates, with the respective concentrations of the inducer. Posteriorly, the plates were maintained in B.O.D. at the temperature of 25± 1ºC and photoperiod of 12 hours.

The mycelial growth was monitored during 48 hours after the incubation in B.O.D, being finalized in this period due to the fact that the Petri® plates of all treatments had their borders struck by the mycelial growth of *R.solani.*

The data were subjected to the variance analysis (($p \leq 0.05$), and when significant, were subjected to the regression analysis, being adopted a level of 5% of significance, using the program ASSISTAT (SILVA; AZEVEDO, 2009).

Results and discussion

The results demonstrated that the treatment of tomato seeds with AS did not interfere significantly on seed germination, incidence of damping-off of seedlings and on seedling length (table 1).

Considering the results of seed germination, we observed that the AS did not present phytotoxic results over the germination; this result is considered ideal, because regarding an inhibitory effect at the germination, it could limit the use of the product as a resistance inducer on the treatment of tomato seeds, fact that was observed by SILVEIRA et al. (2000), when the application of salicylic acid in rice seeds on the concentrations of 0.1; 0.5; 1.0; 10 e 20 μM presented inhibitory effect on germination, being more evident at the concentrations of 10 e 20μM.

In relation to the incidence of damping-off, although the control treatment presented the highest incidence (12.5%), and the treatment with 1.0 mM of AS the lowest (6.25%), we did not observe significant statistical difference among the treatments. Such fact can be related to the low incidence of damping-off occurred in the experiment, which does not exclude its effect on the control of damping-off in conditions of high incidence of the disease.

Just as it occurred in the germination and incidence, the treatment of tomato seeds with AS did not interfere on seedling length; this is positive, because it demonstrates that there was no phytotoxic

Table 1. Seed germination, incidence of damping-off and seedling length of tomato seeds subjected to seed treatment with AS, and to the inoculation of *R.solani*. Dois vizinhos, 2014.

Concentration of AS (mM)	Germination (%)	Incidence (%)	Seedling length (cm)
0	87.50^{ns}	12.50 ^{ns}	8.507^{ns}
0.5	90.00	10.00	8.167
1.0	93.75	6.25	8.532
1.5	91.25	8.75	8.360
2.0	91.25	8.75	8.735

^{ns}non-significant at 5% probability by the F test.

damage over the meristematic tissues, which are responsible for the insertion of new cells in the stem and root apexes (TAIZ e ZEIGER, 2013).

On the other hand, the seed treatment with AS reduced the fresh matter mass (MMF) with the increase of concentration (figure 1). This result can be related to a resistance induction on the seedlings, because the activation of the defense mechanism disfavors the accumulation of mass, in order to form defense compounds. In bean plants, the use of the resistance inducer acibenzolar-S-methyl analogous to the AS, altered the plant metabolism, generating metabolic cost and redirecting of the photoassimilates to invest in defense, though reducing the productivity (KUHN, 2007). HEIL et al. (2000) stated that the production of PR proteins can compete with proteins that are need for basic processes of the plant, being able to compromise its growth and development.

The treatment of tomato seeds with AS under the concentration of 2 mM did not alter significantly the activity of the enzyme phenylalanine ammonia-lyase (FAL) and chitinase, on the other hand, it elevated significantly the activity of the enzyme β-1.3-glucanase (figure 2).

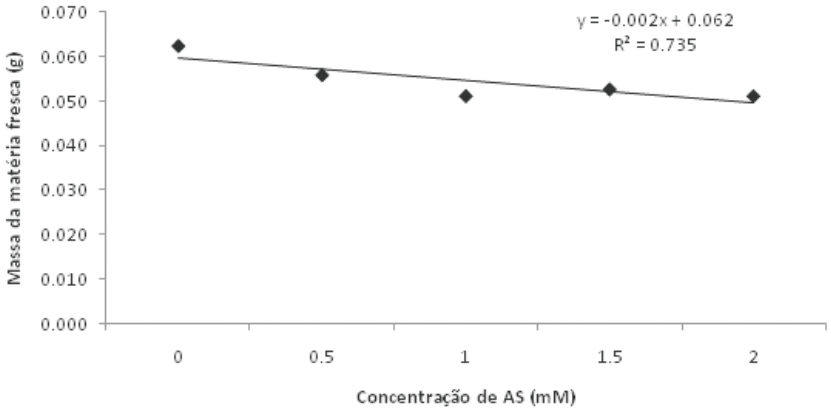

Figure edited in portuguese by the author
(vertical: Fresh matter mass (g) | horizontal: AS concentration (mM))

Figure 1. Fresh matter mass of tomato seedlings subjected to seed treatment with AS, and inoculated with R.*solani.* Dois Vizinhos, 2014.

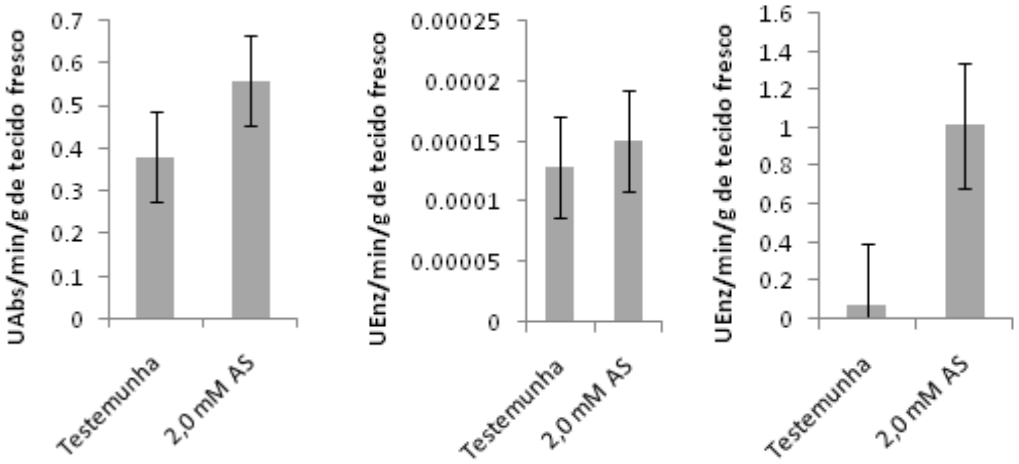

Figure edited in portuguese by the author
(vertical: UAbs/min/g of fresh tissue; UEnz/min/g of fresh tissue; UEnz/min/g of fresh tissue | horizontal: Control; 2.0 mM of AS)

Figure 2. A -Activity of the enzyme phenylalanine ammonia-lyase (FAL), **B –** Activity of the enzyme chitinase, **C –** Activity of the enzyme β-1.3-glucanase, from tomato seedlings subjected to seed treatment with AS. Dois vizinhos, 2014.

This result demonstrates that there was an activation of the defense mechanisms caused by the application of AS in the seed, since there was an increase in the levels of the enzyme β-1.3-glucanase, which is one of the PR proteins that demonstrate the activation of the mechanism of systemic acquired resistance (RSA) (DURRANT and DONG, 2004). Exogenous applications of AS induce the gene expression of proteins involved in the induction of systemic acquired resistance, leading to the proposition of the role of endogenous AS on the resistance to diseases (CHET, 1993), by the expression of proteins related to pathogenicity (PRPs) (CAMPOS, 2009).

Other authors also obtained results related to the AS application in the activity of β-1.3-glucanase; in bean plants, the application of salicylic acid at 0.01 M, at the stage V2, elevated the activity of glucanases and chitinases, which resulted in lower severity of the disease caused by the fungus *Colletotrichum lindemuthianum* (CAMPOS et al., 2009). But as for bean plants treated with AS and infected by *Sclerotinias clerotiorum* **there was an increase on the activity of** β-1.3-glucanase (INOCÊNCIO et al., 2009).

Furthermore, the non observation of activity of the enzymes FAL and chitinase can be related to the fact that in the moment of collection of the vegetal material (14 days, end of experiment), the levels of these enzymes were already similar to the control treatment, and its activation occurred before the time of assessment.

We did not observe direct effect of AS over the mycelial growth of *R.solani*, since in the first assessment (second day after the incubation), in all treatments the mycelium of the fungus had occupied the whole plate, not having a statistic difference among the treatments.

Possibly, this result is related to the concentration studied, since in assessing the effect of AS under more elevated concentrations, it was observed that under the concentration of 10 mM, occurred 100% inhibition of mycelial growth of R. solani (KATARIA et al., 1997). According to Kuć (2001), the resistance inducers can present fungitoxic effect, depending on the concentration used and the pathogen assessed.

However, we suggest new studies that seek to assess more elevated concentrations of AS, or yet different inoculum pressures, or even with another pathosystem, since the AS induces the plant resistance, which can result on potential the control of tomato diseases; besides conducting the collection of vegetal material in different intervals of time during the experiment, which will allow to evaluate the enzymatic behavior of the PRPs.

Conclusion

The application of AS in tomato seeds did not interfere on the incidence of seedling damping-off, but induced the resistance by activating the enzyme β-1.3-glucanase.

The AS does not present effect over the *R.solani in vitro*.

References:

ALVARENGA, M.A.R. Tomate: produção em campo, casa-de-vegetação e em hidroponia. Lavras: Ufla. 2004. 400p.

CAMPOS, A. D; HAMPE, M. M. V; FERREIRA, A. G; ANTUNES, I. F; CASTRO, L. A. S. Indução de resistência sistêmica à antracnose em feijoeiro-comum pela raça delta avirulentade *Colletotrichum lindemuthianum*. Pesquisa Agropecuária Brasileira, Brasília v. 44, n. 1, p. 15-21, 2009. DOI: 10.1590/S0100-204X2009000100003.

CHET, I. Biotechnology in plantdiseasecontrol. 373p. New York: Wiley-Liss, 1993

DURRANT, W.E.; DONG, X. Systemic acquired resistance. Annual Review of Phytopathology, v.42, p.185-209, 2004.DOI: 10.1146/annurev.phyto.42.040803.140421.

GRANDO, V. Batata: principais pragas e doenças. In: CorreioAgrícola, p.10-13.2002.

GUZZO, S.D.; MARTINS, E.M.F. Local and systemic induction of β-1,3-glucanase and chitinase in coffee leaves protected against *Hemileia vastatrix* by *Bacillus thuringiensis*. Journal of Phytopathology, Berlin, v.144, p.449-454, 1996. DOI: 10.1111/j.1439-0434.1996.tb00322.x.

HAMMERSCHMIDT, R.; MÉTRAUX, J. P.; VAN LOON, L. C. Inducing resistance: a summary of papers presented at the first international symposium on induced resistance to plant diseases, Carfu, May 2000. EuropeanJournal of Plant Pathology, Dordrecht, v. 107, p. 1-6, 2001. DOI: A:1008753630626.

HEIL, Martin; BOSTOCK, Richard M., Induced systemic resistance (ISR) against pathogens in the context of induced plant defences.Annals of botany, London, v. 89, p. 503-512, 2002.DOI: 10.1093/aob/mcf076.

HEIL, M; HILPERT, A; WERNER.K; LINSENMAIR, K.E.Reduced growth and seed following chemical induction of pathogen defence: does systemic acquired (SAR) incur allocation costs? Journal of Ecology, Londres, v.88, p.645-654, 2000.DOI: 10.1046/j.1365-2745.2000.00479.x.

INOCÊNCIO, A. P. M.; OLIVEIRA, M. B.; SANTOS, E. M.; NASCIMENTO, L. B.; ALVES; E. B.; SOARES, D. A.; LOBO JR., M.; SILVA, S. P.;Indução de resistência em planta de feijoeiro infectada por Sclerotinias clerotiorum.In: 25º Congresso Brasileiro de Microbiologia.ID:2096-1. Porto de Galinhas, Nov. 2009

KATARIA, H.R; WILMSMEIER, B. Efficacy of resistance inducers, free-radical scavengers and an antagonist strain of Pseudomonas fluorescens for control of *Rhizoctonia solani* AG-4 in bean and cucumber. Plant Pathology, London, v.46, p.897-909, 1997.DOI: 10.1046/j.1365-3059.1997.d01-75.x.

KUĆ, J. Concepts and direction of induced systemic resistance in plants and its application. European Journal of PlantPathology, v.107, p.7-12, 2001. DOI: 10.1023/A:1008718824105.

KUHN, O.J. Indução de resistência em feijoeiro (*Phaseolus vulgaris*) **po**r acibenzolar-S-metil e Bacilluscereus: aspectos fisiológiocos, bioquímicos e parâmetros de crescimento e produção. 2007. 140p. Tese (Doutorado) - Escola Superior de Agricultura Luiz de Queiroz, Piracicaba.

LIU, J; ZONG, Y; WISNIEWSKI, M; DROBY, S; TIAN, S; NORELLI, J; HERSHKOVITZ, V. effect of heat treatment on inhibition of Moniliniafructicola and induction of disease resistance in peach fruit. Postharvest Biology and Technology, v.65, p.61-68, 2012.DOI: 10.1016/j.postharvbio.2011.11.002.

MARTINEZ, C.; BACCOU, J. –C.; BRESSON, E.; BAISSAC, Y.; DANIEL, J. –F.; JALLOUL, A.; MONTILLET, J. –L.; GEIGER, J. –P.; ASSIGBETSÉ, K.; NICOLE, M. Salicylic acid mediated by the oxidative burst is a key molecule in local and systemic responses of cotton challenged by an avirulent race of X*anthomonas campestris* pvmalvacerum. Plant Physiology, Bethesda, v. 122, p. 757-766, 2000. DOI: 10.1104/pp.122.3.757.

MAZARO, S.M.; WAGNER JUNIOR, A.; SANTOS, I.; CITADIN, I.; POSSENTI, J.; GOUVEA, A. Controle do tombamento de plântulas de beterraba e tomate pelo tratamento de sementes com quitosana. Pesquisa Agropecuária Brasileira, Brasília, v. 44, n. 11, p. 1424-1430, 2009. DOI: 10.1590/S0100-204X2009001100008.

MORAES, W. C. Controle alternativo de fitopatógenos. Pesquisa Agropecuária Brasileira, Brasília, v. 27, p. 175-190, 1992.

SECRETARIA DA AGRICULTURA E DO ABASTECIMENTO DO PARANÁ - SEAB. Análise da conjuntura agropecuária: safra 2008/09. Disponível em: <http://www.seab.pr.gov.br/arquivos/File/deralPrognosticos/olericultura_0809.pdf>. Acesso em: 10 maio 2011.

SILVA, F. A. S.; AZEVEDO, C. A. V. Principal ComponentsAnalysis in the Software Assistat-StatisticalAttendance. In:World congress on computers in agriculture, 7, Reno-NV-USA: American Society of Agricultural and Biological Engineers, 2009.

SILVEIRA MAM; MORAES DM; LOPES NF. 2000. Germinação e vigor de sementes de arroz (Oryza sativa L.) tratadas com ácido salicílico. Revista Brasileira de Sementes. v.22, n.2, p.145-152,2000.

STADNIK, M. Indução de resistência a oídios.In: CONGRESSO PAULISTA DE FITOPATOLOGIA, Campinas. Anais do Congresso Paulista de Fitopatologia, v. 23, p. 176-181. 2000.

STEPHENS, C.T.; POWELL, C.C.; SCHMITTHENNER, A.F.A method of evaluating post-emergence damping-off pathogens of breeding plants. Phytopatology, Lancaster, v.71, p. 1125-1128, 1981. DOI: 0031-949x/11122504/$03.00/0.

TAIZ, L; ZEIGER, E. Fisiologia vegetal. 5 ed. Porto alegre: Artemed, 2013, p.918.

WIRTH, S.J.; WOLF, G.A.; Micro-plate colourimetric assay for endo-acting cellulose, xylanase, chitinase, 1,3-β-glucanase and amylase extracted from forest soil horizons. Soil Biology and Biochemistry, v.24, p.511-519, 1992.DOI: 10.1016/0038-0717(92)90074-8.