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Glyceolin and phaseolin induction in soybean and beans as a function of biocontrol agents application

Indução fitoalexinas em cotilédones de soja e hipocótilos de feijão em função da aplicação de agentes de biocontrole

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Resistência induzida, Gliceolina, Faseolina.

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Induced resistance, Glyceolin, Phaseolin.

Abstract

Induction of plant resistance is a tool that can be incorporated into the integrated management of diseases of cultivated plants. In this way, this work aims to evaluate the elicitor action of biocontrol agents in the induction of glyceolin. In this study were used concentrations about 0; 0.5; 1; 1.5 and 2% diluted in water; spore suspension of Trichoderma asperellum BV10, Bacillus subtilis BV02 and Bacillus amyloliquefaciens BV03 microorganisms. To determine the glyceolin, cotyledons were grown in sand, weighed and cut in longitudinal section on the lower surface. Subsequently, these were deposited 50 µL of the concentrations in the cuts and the extraction performed in H2O and concentration determined by absorbance at wavelength 285nm. The results were submitted to analysis of variance and compared by the regression test ($p < 0.05$). When measuring the glyceolin in soybean cotyledons, there was an increase in the accumulation of this phytoalexins as the concentration of B. subtilis BV02 cells increased. The 4% concentration of B. subtilis BV02 promoted 237% more glyceolin accumulation in soybean cotyledons than the control. The accumulation of glyceolin was increased as the concentration of T. asperellum BV10 cells increased, so that 4% concentration promoted 228% more glycerol accumulation than the control. Increasing the concentration of Bacillus amyloliquefaciens BV03 cells increased the accumulation of this phytoalexins. The 4% concentration promoted 129% more accumulation of glycerol in soybean cotyledons than the control. The microorganisms T. asperellum, B. subtilis and B. amyloliquefaciens are indicated as activators of resistance mechanisms of soybean plants.

Resumo

A indução de resistência de plantas a patógenos é uma ferramenta que pode ser incorporada ao manejo integrado de doenças de plantas cultivadas. Assim, o objetivo deste trabalho foi avaliar a ação elicitora de agentes do biocontrole na indução de gliceolina. Para isto foi utilizado as concentrações 0,0; 0,5; 1,0; 1,5; 2,0 e 4,0%, do concentrado de esporos, diluídas em água, da suspensão de esporos dos microrganismos Trichoderma asperellum BV10, Bacillus subtilis BV02 e Bacillus amyloliquefaciens BV03. Para determinação da gliceolina, cotilédones foram cultivados em areia, pesados e cortados em secção longitudinal na superfície inferior. Posteriormente, estes foi depositado 50 µL das concentrações nos cortes e a extração realizada em H2O e concentração determinada por absorbância no comprimento de onda 285nm. Os resultados foram submetidos à análise de variância e comparados pelo teste de regressão ($p<0,05$). Ao mensurar a gliceolina em cotilédones de soja, houve aumento no acúmulo de fitoalexina à medida que aumentou a concentração de células B. subtilis BV02. A concentração 4,0% de B. subtilis BV02 promoveu 237% mais acúmulo de gliceolina em cotilédones de soja que a testemunha. O acúmulo de gliceolina foi incrementado conforme se aumentou a concentração de células de T. asperellum BV10, de forma que concentração 4,0% promoveu 228% mais acúmulo de gliceolina que a testemunha. Ao aumentar a concentração de células Bacillus amyloliquefaciens BV03 aumentou-se o acúmulo desta fitoalexina. A concentração 4,0% promoveu 129% mais acúmulo de gliceolina em cotilédones de soja que a testemunha. Os microrganismos Trichoderma asperellum, Bacillus subtilis e Bacillus amyloliquefaciens são indicados como ativadores de mecanismos de resistência de plantas de soja.

INTRODUCTION

Plants are constantly attacked by many pathogens with high destructive potential, and respond to these activating their defense genes, producing reactive oxygen species (ROS), synthesizing proteins (PR) related to pathogenesis, reinforcing the cell wall and producing antimicrobial compounds. Among the antimicrobial compounds are phytoalexins, induced during the attack of a plant pathogen or exposure of the plant to an elicitor, making an important part of the plant defense repertoire (PEDRAS et al., 2008; PEDRAS et al., 2009).

A wide spectrum of phytoalexins has been described in several plant species, induced by different pathogens (AHUJA et al., 2012; IRITI, FAORO, 2009). The synthesis of phytoalexins is one of the fastest metabolic responses of plants to activate external elicitors, due to the analysis of these substances represents an important tool of studies about resistance induction events through inductive or elicitor agents of biotic or abiotic origin (PEITER-BENINCA et al.; 2008; VIECELLI et al., 2009).

Gliceolin are often reported on induction studies and can be considered as indicator plants of this type of analysis, because they are easily measured and they have an efficient response of eliciting agents. Phytoalexins play an important role in plant-pathogen interaction in response to infection and application of resistance inducers (FRANZENER et al., 2000). A variety of elicitors, being proposed in the literature, among them, are biotic agents, such as microorganisms, that can activate plant defense mechanisms (STANGARLIN and PASCHOLATI, 1994). Filtrated phytopathogenic, non-pathogenic and saprobic fungi are able to activate the defense system, since they have molecules present as proteins, oligosaccharides, oligopeptides, toxins and others that function as a signature of recognition of the microorganism that are recognized by protein receptors, present in the cell membrane of the plant cell (DERERY et al., 2012). One way to identify the resistance-inducing action of plant pathogens is observe the biochemical changes occurring in the plant, such as the accumulation of phytoalexins (CHOYDHANRY; JOHRI, 2007).

Considering biocontrol agents capacity to control diseases of cultivated plants, this work aims to evaluate the accumulation of glycerol as a function in application of *Trichoderma asperelum* BV10, *Bacillus subtilis* BV02 and *Bacillus amyloliquefaciens* BV03 in soybean cotyledons.

MATERIAL AND METHODS

The phytoalexin induction assay in soybean cotyledons was conducted in a greenhouse and phytopathology laboratories at the University of Rio Verde, Fazenda Fonte do Saber, Rio Verde (GO).

The experiment was conducted in a completely randomized design OF 3 x 5 factorial arrangement, totaling 15 treatments, with six replicates, five cotyledons per replicate. Factor A corresponds to three species of biocontrol agentes: *T. asperellum* BV10, *B. subtilis* BV02 and *B. amyloliquefaciens* BV03, and factor B corresponds to 0.0; 0.5; 1.0; 2.0 and 4.0% of biocontrol agents.

To obtain cotyledons, soybean seeds were sown in sterilized sand and kept in a greenhouse. From 7 to 10 days after sowing the cotyledons were detached from the seedlings, washed in sterilized distilled water, dried and cut in an approximate 1 mm thick and 6 mm in diameter on the abaxial surface. The cotyledons were weighed and placed in a Petri dish containing filter paper moistened with sterile distilled water. A 50 µl aliquot of the concentrations of the biocontrol agents was deposited on the cuts of each cotyledon. Petri dishes were kept at 25 °C in the dark. After 20 hours, the cotyledons were transferred to test tubes containing 15 mL of sterilized distilled water and allowed to stir for 1 hour to extract the formed glycerol, which was measured in a spectrophotometer using the wavelength of 285 nm (AYERS et al., 1976; ZIGLER; PONTZEN, 1982). The results were expressed as units of absorbance per gram of fresh weight (ABS.gpf^{-1}).

To determine the phaseolin, bean seeds were sowed in polyethylene trays with sterilized sand. The trays were stored in a B.O.D. incubator, in the dark to promote the hypochlotyping. Eight days after sowing, the hypocotyls were detached, considering 5 cm of the middle third and washed in deionized water. The hypocotyls, four per repetition, were arranged in a gerbox, containing 2 germitest papers, moistened with deionized water. The treatments were sprayed on the hypocotyls with hand atomizer and the gerbox boxes were kept in the dark for 48 hours. After the 48-hour period, the hypocotyls were weighed and transferred to test tubes containing 5 mL of ethanol and conditioned at 4 °C for 48 hours. For the extraction of formed phytoalexin, the tubes were shaken in an orbital shaker table for 1 hour and the determination was made in a spectrophotometer using wavelength of 280 nm. The results were expressed as absorbance units per gram of fresh weight (ABS.gpf-1) (DIXON et al., 1983).

The data were submitted to normality test of Shapiro Wilk, assuming significance by regression test ($p < 0.05$).

RESULTS AND DISCUSSION

When applying *T. asperellum* BV10, *B. subtilis* BV02 and *B. amyloliquefaciens* BV03, there is an increase of glycerol accumulation in soybean cotyledons as the concentration of biocontrol agents is increased. The 4.0% concentration of *B. subtilis* BV02 promoted a 205% increase in glycerol accumulation in soybean cotyledons, compared to application of distilled water (Figure 1).

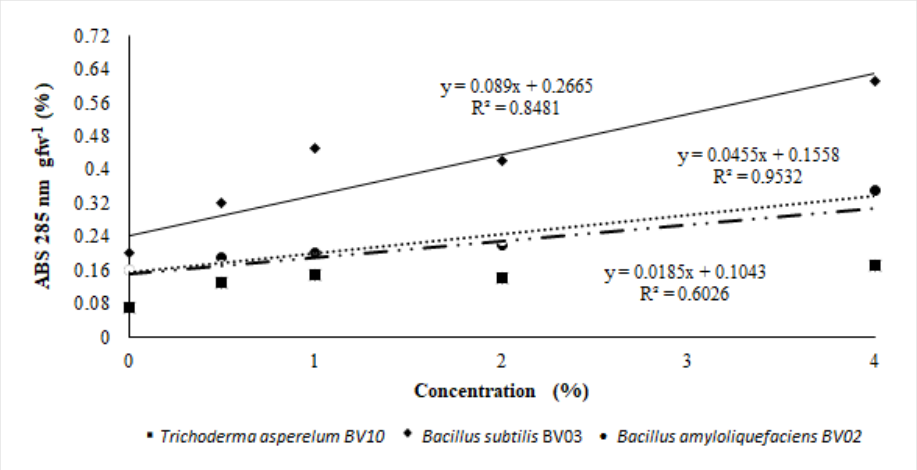

Figure 1. Glycerol accumulation in soybean cotyledons due to application of concentrations of biocontrol agents.

The determination of secondary compounds involved in the defense of plants to pathogens, such as phytoalexins, are fast and efficient tools for the identification of inducing, eliciting and plant resistance agents. When determining the accumulation of glycerol in soybean cotyledons, it was observed that the tested biocontrol agents, *T. asperellum* BV10, *B. subtilis* BV02 and *B. amyloliquefaciens* BV03 have eliciting action when applied to soybean cotyledons, inducing accumulation of glycerol. Jungues et al. (2016) also reported the potential of *B. subtilis* in inducing resistance of bean plants to anthracnose, increasing the activity and rate of peroxidase synthesis, in addition to increasing the rate of β-1,3-glucanase synthesis and reducing the severity and the progress of the disease, reinforcing that the species of *B. subtilis* can be used as inducer of resistance of plants to pathogens. Silva et al. (2011) reported that *T. asperellum* presents great potential for efficiency as a plant growth promoter and as an anthracnose resistance inducer in cucumber, conferring protection from 56.36 to 87.30%.

When measuring phaseolin in bean hypocotyls, it can be observed in Figure 2 that increasing the concentration of *B. amyloliquefaciens* BV03 increases the accumulation of this phytoalexins. The 1.70% concentration of *B. amyloliquefaciens* BV03 promoted 11.20% more accumulation of phaseolin in bean hypocotyls than distilled water (control), showing an increase of 1.25 nm.gpf $^{-1}$ (Figure 2).

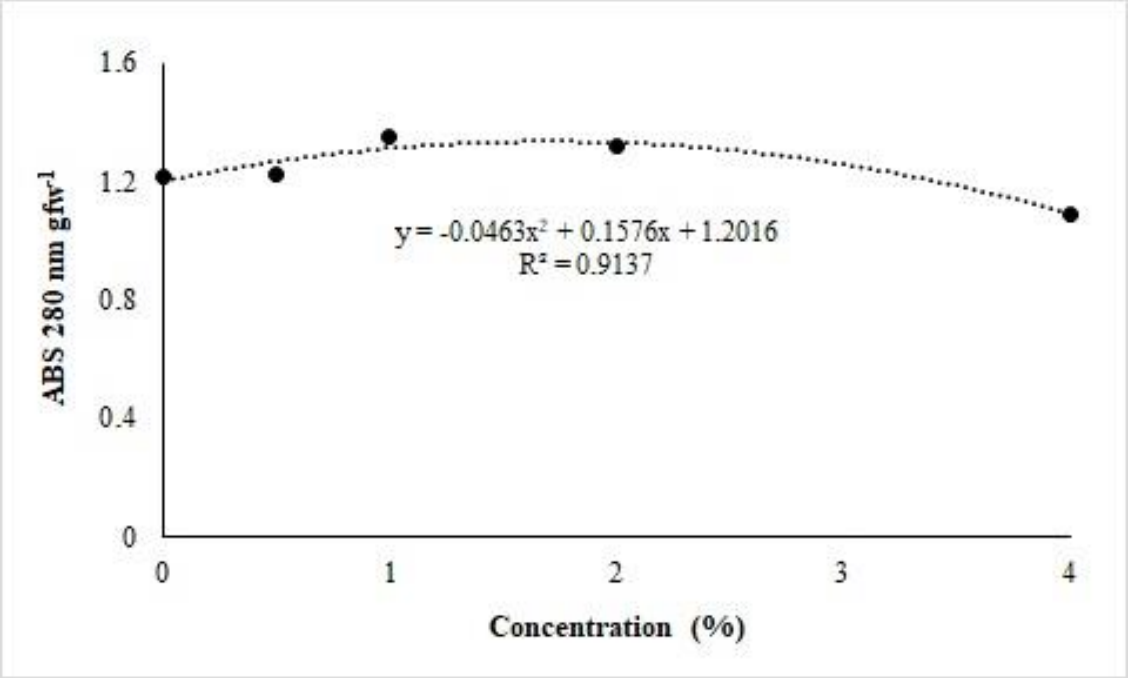

Figure 2. Accumulation of phaseolin in bean hypocotyls as a function of the application of Bacillus amyloliquefaciens BV03 concentrations.

The pulverization application of bean hypocotyls with concentrations of *T. asperellum* BV10 and *B. subtilis* BV02 did not influence the accumulation of phaseolin (Table 1).

Table 1. Phaseolin accumulation at bean hypocotyls as a function of *Bacillus subtilis* BV02 and *Trichoderma asperellum* BV10 concentration.

Concentration (%)	Biocontrol agent	
	B. subtilis BV02	*T. asperellum* BV10
	ABS 280 nm gfw^{-1} (%)	
0,0	0,92^{ns}	1,26 ^{ns}
0,5	0,89	1,23
1,0	1,01	1,19
2,0	1,01	1,15
4,0	1,05	1,07
CV (%)	24	14

ns = not significant

Isolated biocontrol agents *T. asperellum* BV10 e *B. subtilis* BV02 did not promote the induction of phaseolin in bean hypocotyls. According to Solino et al. (2017), the elicitor can act extensively on resistance induction, being the mechanism of several species of plants or act specifically in one or more species.

B. amyloliquefaciens BV03, on evaluated concentrations, promoted induction of glycerol in soybean and phaseolin in bean, indicating a broad spectrum of action as elicitor agent of resistance plants mechanism to pathogens. *Bacillus* microorganisms produce several inter and extracellular components that have an eliciting action on the resistance of plants to pathogens (ONGENA et al., 2007; YANG et al., 2011; YI et al., 2016) . Wu et al. (2018), found *B. amilolyquefaciens* strains with suppressed extracellular components promoted increased levels of salicylic acid, jasmonic acid and ethylene, involved in translation and signaling of plant resistance, to a lesser extent than strains with extracellular components, indicating the components found in this are responsible for the activation of plant resistance mechanisms (HUANG et al., 2012; YI et al., 2016; WU et al., 2018).

CONCLUSION

Biocontrol agents *T. asperellum* BV10, *B. subtilis* BV02 e *B. amyloliquefaciens* BV03 promote gliceolin indution on soybean cotyledons.

B. amyloliquefaciens BV03 promote phaseolin indution on bean hypocotyls.

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