

## Induction of Phytoalexins Gliceoline and Proteins Related to Defense in Soybean Cotyledon Treated With Yeast

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### Abstract

Yeasts can induce mechanisms of plant resistance due to compounds with eliciting characteristics, so the aim of this work was to evaluate the effect of yeast on the induction of phytoalexins gliceoline, peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase from soy cotyledons. To determine the defense enzymes, soybean seeds were sown and the cotyledons treated with sterile distilled water, *Cryptococcus laurentii* (AH 03-1), *Pichia guilliermondii* (AH 16-2), *Rhodotorula glutinis* (AH 14-3), *Sporidiobolus johnsonii* (AH 16-1) and *Zygoascus hellenicus* (AH 14-1). Biochemical analyzes of the formation of phytoalexins and the activity of the enzymes peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase and total proteins were performed. For phytoalexins glycerolins the yeasts *Cryptococcus laurentii* (AH 03-1) and *Zygoascus hellenicus* (AH 14-1) promoted an increase of 83.65% and 78.75% in the formation of this compound. *Cryptococcus laurentii* (AH 03-1) increased peroxidase activity by 36.84%, while for polyphenoloxidase, the *Pichiaguilliermondii* e yeasts (AH 16-2), *Rhodotorula glutinis* (AH 14-3), *Sporidiobolus johnsonii* (AH 16-1) and *Zygoascus hellenicus* (AH 14-1), increased the activity of this enzyme by 33.33%, 28.00%, 33.33% and 33.33%, respectively. For phenylalanine ammonia-lyase, *Cryptococcus laurentii* (AH 03-1) and *Zygoascushellenicus* (AH 14-1) promoted an increase of 75.57% and 78.86%, respectively, in their activity. The results demonstrate the potential of yeasts studied in the induction of phytoalexins glyceolins and in the activity of peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase in soybean cotyledons.

**Keywords:** *Glycine max*, induction of resistance, peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase

### 1. Introduction

The soybean (*Glycine max* (L.) Merrill), is a very important crop (Hirakuri & Lazzarotto, 2014), host of several pathogens that affect both the productivity and quality of the final product (Juhász et al., 2013).

In order to reduce disease losses, control methods are used, some of which are classified as alternative (Carneiro et al., 2011), where resistance induction is involved (Schwan-Estrada et al., 2008) responsible for the activation of latent mechanisms of plant defense against pathogens by treatment with biotic or abiotic elicitor molecules (Pascholati, 2011).

Among the many resistance inducers, we can mention the use of fungi (Medeiros et al., 2014) as yeasts (Mello et al., 2011). Several defense mechanisms can be activated such as enzymes, such as peroxidase, important in cell lignification, polyphenoloxidase responsible for modifying phenols in toxic quinones and phenylalanine ammonia-lyase, predecessor of the synthesis of defenylpropanoids, involved in plant defense (Stangarlin et al., 2011), And one of the most important in the biosynthesis of lignin and phytoalexins (Kalaiarasan, 2009).

Another example would be the production of phytoalexins (Peiter-Beninca et al., 2008), which are antimicrobial compounds (Paschoalati & Dalio, 2018) responsible for inhibiting germination and mycelial growth (Pascholati, 2011).

In view of the above, the objective of this work was to investigate the potential of yeast in phytoalexin-glycine oxidase induction on the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase in soybean cotyledons treated with different yeasts.

## 2. Materials and Methods

The assay for the evaluation of the phytoalexins and glycinolins induction and enzymatic activity in soybean cotyledons was conducted at the Phytopathology Laboratory of the State University of the West of Paraná (Unioeste), *Campus* of Marechal Cândido Rondon - PR, the same laboratory being the supplier of used yeasts.

The experimental design was completely randomized with six treatments (sterile distilled water, *Cryptococcus laurentii* (AH 03-1), *Pichia guilliermondii* (AH 16-2), *Rhodotoru laglutinis* (AH 14-3), *Sporidiobolus johnsonii* (AH 16-1) and *Zygoascus hellenicus* (AH 14-1)), and four replicates.

Soybean seeds of the cultivar M5947 IPRO were disinfested in 70% alcohol and hypochlorite (3:1), then washed with running distilled water and seeded in plastic trays containing autoclaved sand at 120 °C for 2 h. Daily watering was carried out with distilled water and kept in the growth chamber for about 10 days.

When freshly opened, the totally healthy cotyledons were detached, washed with distilled water, dried and in the abaxial region a section was made using a scalpel. The cut cotyledons were placed into Petri dishes containing filter paper moistened with sterile distilled water and five cotyledons were distributed per plate with the cuts facing upwards. A 20 µL aliquot of the treatments was applied on each cut.

The petri dishes were kept dark in BOD at 25 °C for 20 h. After, the cotyledons were transferred to plastic vials containing 10 mL of sterile distilled water. The flasks remained at 150 rpm on an orbital shaker for 1 hour for extraction of phytoalexin gliceoline.

Supernatant readings were performed at 285 nm in spectrophotometer and the five cotyledons of each replicate were weighed on analytical balance.

These same cotyledons were used with 4 mL of 0.01 M serum phosphate (pH 6.0) and the liquid nitrogen in a porcelain mortar, the homogenate being centrifuged at 6,000 g for 20 minutes at 4 °C and the supernatant stored at -20 °C for the accomplishment of the biochemical analyzes.

For the peroxidase activity the methodology described by Hammerschmidt et al. (1982) was used by mixing 100 µL of the supernatant and 900 µL of the enzyme substrate with spectrophotometer readings for 2 minutes at 470 nm.

To determine the polyphenoloxidase activity, the Duangmal and Apenten method (1999) was used, creating 900 µL of substrate for one enzyme and 100 µL of supernatant. As readings, they were made in a spectrophotometer at 420 nm for 2 minutes. Both paraperoxidase and parapolyphenoloxidase, were expressed as absorbance  $\text{min}^{-1} \text{mg}^{-1}$  protein.

For the activity of the phenylalanine ammonia-lyase was used the colorimetric quantification method of the trans-cinnamic acid released from the substrate, according to Umesha (2006), where 50 µL of the supernatant was mixed in 450 µL of Tris-HCl 0.025 M buffer pH 8.8 and 500 µL of 0.05 M phenylalanine solution were added and the mixture was incubated in a water bath at 40 °C for 2 h, and then 60 µL of 5 M HCl was added to stop the reaction. The spectrophotometer was read at 290 nm. The values were obtained by the difference of the sample and control.

The methodology of Bradford (1976) was used to determine the total important proteins in the calculation of the enzymes. 200 µL of the supernatant, 600 µL of 0.01 M phosphate buffer (pH 6.0) and 200 µL of Bradford reagent were added, the latter being added under stirring. After shaking, the samples were incubated for 10 minutes and read at 595 nm in a spectrophotometer. The absorbances obtained were plotted on a standard curve of bovine serum albumin.

The datas were submitted to analysis of variance and the means were compared by the Tukey test at 5% probability of error using the free software Genes (Cruz, 2013).

## 3. Results and Discussion

For phytoalexins, according to Figure 1, *Cryptococcus laurentii* yeasts (AH 03-1) and *Zygoascus hellenicus* (AH 14-1) were statistically different from the water, with respective phytoalexin values of 83.65% and 78.75% compared to water.

In a study accomplished by Stangarlin et al. (2010) using yeasts *S. cerevisiae* and *S. boulardii* in soy cotyledons, the authors found higher phytoalexin induction values for *S. boulardii*, concluding that it is an inducer, but

according to the same authors, this does not exclude activity eliciting of *S. cerevisiae*, since this yeast presents components of the cell wall that can be inactivated with autoclave, which may justify the lowest verified induction.

Working with sorghum mesocotyls, Stangarlin et al. (2010) also verified an inductive effect by *S. boulardii* yeasts, supposedly due to metabolites released by them.

Wulff and Pascholati (1998) studying phytoalexins in sorghum mesocotyls observed the increase of the measurement of this compound using as *S. cerevisiae* yeast.

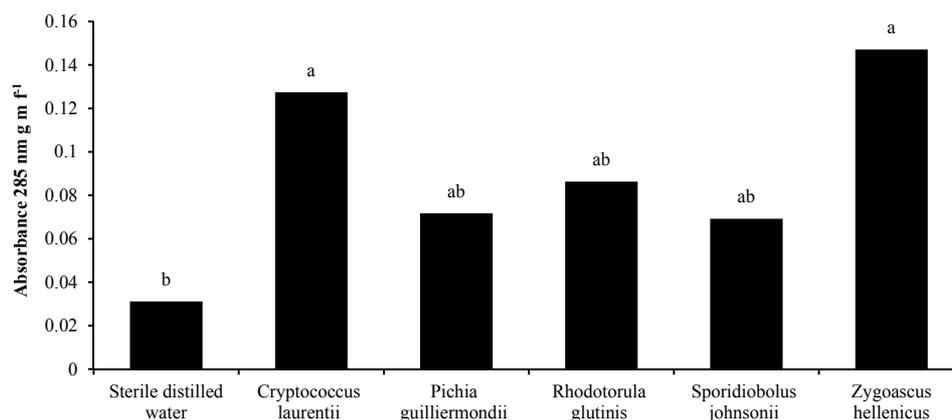


Figure 1. Induction of phytoalexin glycine in soybean cotyledons (*Glycine max*) with different yeasts (*Cryptococcus laurentii* (AH 03-1), *Pichia guilliermondii* (AH 16-2), *Rhodotorula glutinis* (AH 14-3), *Sporidiobolus johnsonii* (AH 16-1) and *Zygoascus hellenicus* (AH 14-1)). The averages of the same column letter did not differ statistically by the Tukey's test ( $p < 0.05$ ). CV = 18.37

In relation to a peroxidase activity in soybean cotyledons only one *Cryptococcus laurentii* yeast (AH 03-1) appeared different from water, with an increase of 36.84% compared to it, according to Figure 2.

According to Viecegli et al. (2010), changes in the activity of the peroxidase enzyme refers to the susceptibility or resistance response to numerous patosystems. Resende et al. (2007) complements the claim that this enzyme is responsible for catalyzing an oxidation of phenolic alcohols to lignin, which causes the largest company against toxins released by pathogens.

In a study carried out by Formentini (2012), the initial activity of this enzyme may be related to the pre-disposition resistance. This same author states that the initial activity of this enzyme may be related to the pre-disposition to resistance and according to Stangarlin et al. (2011) the modification of the activity of this enzyme can indicate induction of resistance since the enzymes act preventively for the health of the plant, that is, making it impossible for the pathogen to transpose the cell wall.

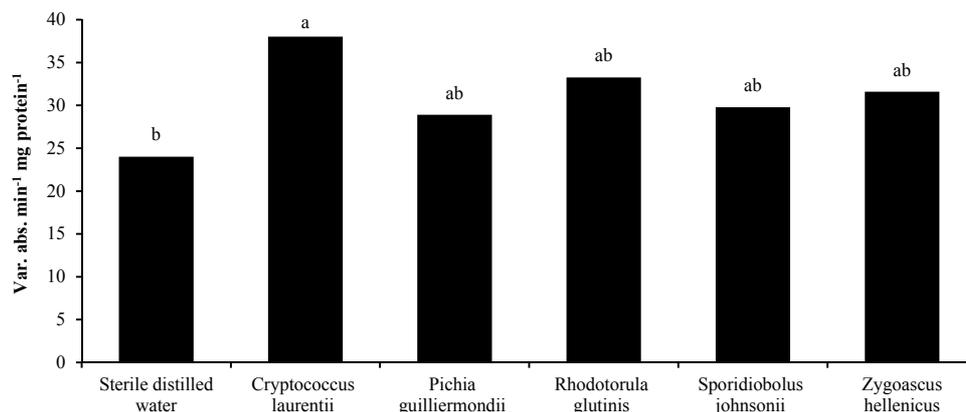


Figure 2. Peroxidase activity in soybean cotyledons treated with different yeasts (*Cryptococcus laurentii* (AH 03-1), *Pichia guilliermondii* (AH 16-2), *Rhodotorula glutinis* (AH 14-3), *Sporidiobolus johnsonii* (AH 16-1) and *Zygoascus hellenicus* (AH 14-1)). \* Averages followed by the same lowercase letter in the column do not differ statistically by the Tukey test ( $p < 0.05$ ). CV = 14.92

For polyphenoloxidase, the activity was more significant in cotyledons treated with *Pichia guilliermondii* yeasts (AH 16-2), *Rhodotorula glutinis* (AH 14-3), *Sporidiobolus johnsonii* (AH 16-1) and *Zygoascus hellenicus* (AH 14-1), which showed an increase of 33.33%, 28.00%, 33.33% and 33.33%, respectively, in the activity of this enzyme compared to the water treatment (Figure 3).

In a study carried out by Khalid (2013), using *S. cerevisiae* yeast in bean plants affected by *Sclerotium rolfsii*, an increase of peroxidase and polyphenoloxidase was observed and consequently the reduction of the disease due to the induction of resistance mechanisms.

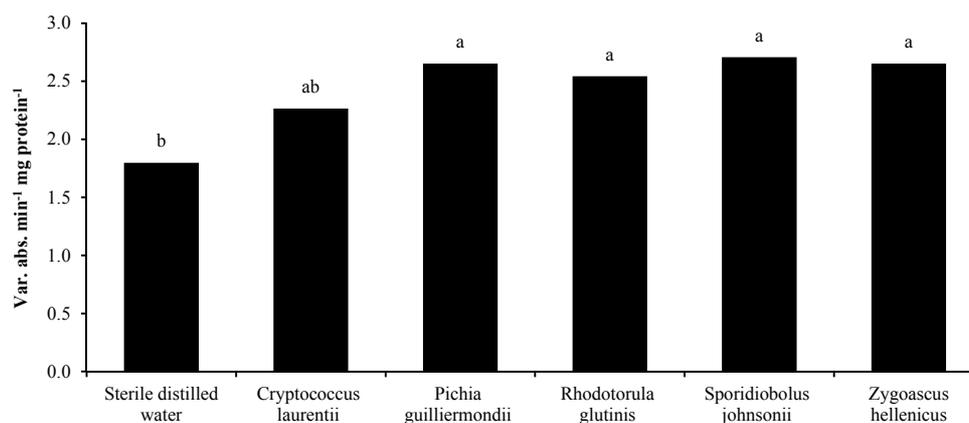


Figure 3. Activity of polyphenoloxidase in soybean cotyledons together with different yeasts (*Cryptococcus laurentii* (AH 03-1), *Pichia guilliermondii* (AH 16-2), *Rhodotorula glutinis* (AH 14-3), *Sporidiobolus johnsonii* (AH 16-1) and *Zygoascus hellenicus* (AH 14-1)). The averages of the same column letter did not differ statistically by the Tukey's test ( $p < 0.05$ ). CV = 12.33

According to Figure 4, for the activity of phenylalanine ammonia-lyase the *Pichia guilliermondii* yeasts (AH 16-2), *Rhodotorula glutinis* (AH 14-3) and *Sporidiobolus johnsonii* (AH 16-1) were statistically equal to water, whereas the yeasts *Cryptococcus laurentii* (AH 03-1) and *Zygoascus hellenicus* (AH 14-1) presented an increase of 75.57% and 78.86%, respectively, purchased from the water treatment, being statistically different.

Klessig and Malamy (1994) say that the activity of the phenylalanine ammonia-lyase enzyme prepares precursors for the biosynthesis of lignin and other phenolic compounds that are deposited as a reaction to

infection, a fact that demonstrates the importance of the activity of this enzyme and how much it can contribute in the induction of resistances.

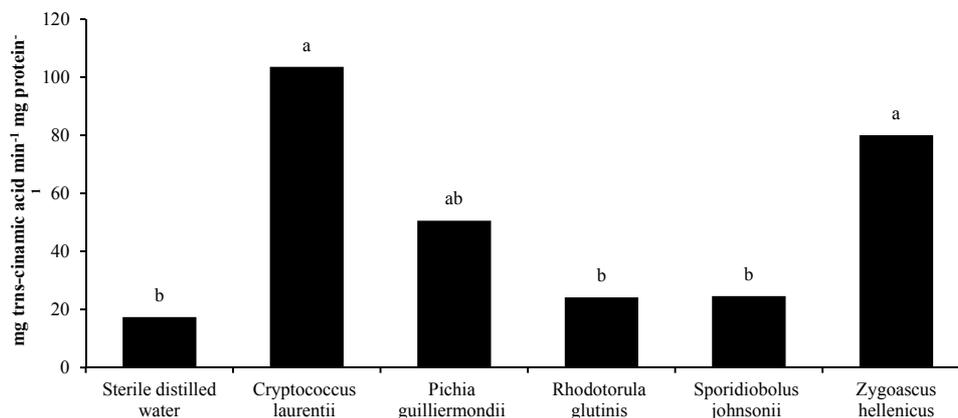


Figure 4. Activity of phenylalanine ammonia-lyase in soybean cotyledons treated with different yeasts (*Cryptococcus laurentii* (AH 03-1), *Pichia guilliermondii* (AH 16-2), *Rhodotorula glutinis* (AH 14-3), *Sporidiobolus johnsonii* (AH 16-1) and *Zygoascus hellenicus* (AH 14-1)). \* Averages followed by the same lowercase letter in the column do not differ statistically by the Tukey test ( $p < 0.05$ ). CV = 36.36

According to Barros et al. (2010), the biofertilizers can alter the systemic resistance induced and the acquired systemic resistance, being that in its composition are the yeasts, which demonstrates the existence of inductive potential of resistance present in them, which Pascholati (1998) also describes the existence of eliciting activity in yeast.

Piccinin et al. (2005) observed studying *S. cerevisiae* properties in resistance induction considering this yeast an elicitor. In a study by Fialho et al. (2010) using *S. cerevisiae* yeast, the production of antimicrobial volatile organic compounds, based on alcohols and esters, has also been identified and may have action on defense mechanisms, indicating the potential of yeasts in the resistance induction.

Stangarlin and Pascholati (1994) state that yeasts promote the activation of plant defenses due to elicitation by compounds present in them and Gouvea et al. (2007) also report the yeast resistance induction capacity.

The increase of the activity of certain enzymes caused by the yeasts can be positive, since according to Romeiro and Garcia (2009), at the moment which the plant is exposed to a certain elicitor preventively, that is, before having contact with a certain pathogen, its tissues present a rapid and efficient reaction to the attempt to colonize the pathogen, which is advantageous.

#### 4. Conclusion

Yeasts are able to induce the activity of phytoalexins gliceolines, peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase in soy cotyledons revealing the action of elicitors present in these fungi.

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