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# Defense Activation In Soybean By The Action Of Amino Acids And Micronutrient Against *Phakopsora pachyrhizi*

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## Abstract

The soybean has a greatest expression among the cereals in the world, widely used in animal and human food, through derivatives. However, Asian rust being one of the major diseases of this crop, is responsible for raising production costs and causing economic damage to the producer. The objective of this work was prove the resistance induction in soybean from the seed treatment based on amino acids and micronutrients (Packseed®). The experiment was carried out in greenhouse at the Federal Tecnologic University – Parana, Campus Dois Vizinhos. The treatments were a product based on amino acids and micronutrients (Packseed®) + conventional seed treatment (insecticide imidacloprid (0.345g of i.a.) + thiodicarb (1.035g of i.a.) fungicide carbedazim (0.75g of i.a.) and polymer (1,5mL)) in comparison to the control, where was used only the conventional seed treatment, both in 100 grams of soybean seeds. The cultivar used was 5909, sowed in 10 liter pots, at a sowing rate of 10 seeds per pot. The plant material was collected after the emergency (0 hours) and an interval of 24, 48, 96 and 192 hours. For the collection, 2 cotyledons/healthy and uninjured leaves were collected to perform the biochemical analyzes. The results demonstrate the product based on amino acids and micronutrients has the potential to induce resistance by the activation of enzyme FAL, through activating the defense route of the phenylpropanoids in the plant.

**Key words:** resistance induction, leaf fertilizer, seed treatment

## INTRODUCTION

The soybean (Glycine max (L.) Merril) is one of the most cultivated oleaginous in the world, presenting a significant increase on the production every year due the importance in the economic market, either to food production, biodiesel, among other derivatives (EMBRAPA SOJA, 2014). However, some factors limit the production increase, of them stand out the numerous diseases that affect the culture.

The Asian rust caused by the fungus Phakopsora pachyrhizi H. Sydow&Sydow, is one of the major soybean diseases (YORINORI *et al*., 2005). This fungus was first detected in the USA in 2004 (Schneider *et al*., 2005), and in Brazil has been causing damages since 2001/02 (YORINORI *ET AL*., 2005; GODOY *ET AL*., 2009). It has the potential to reduce yields from 30% to 75% in moderate infections in soybean crop, in the absence of control measures losses of yield up to 90% of production have been reported (BROMFIELD, 1984; HARTMAN *ET AL*., 2015, KUMUDINI *et al*., 2008; YORINORI *ET AL*., 2005).

The typical symptoms of these disease are small tan lesions on the abaxial surface of soybean leaves, these lesions are associated with leaf chlorosis, disease that can result in a reduced number of pods, a lower oil content and higher rates of seed abortion (BROMFIELD, 1984, Hartman *et al*., 2015). The main form of control is still the use of fungicides, however, has raised production costs and reduced profit margins of producers. In Brazil, fungicide application costs and income losses to control the disease have averaged US $ 1.98 billion/year from 2004 to 2014 (GODOY *et al*., 2016). Thus, the development of new strategies in the control of disease is of utmost importance.

Among these techniques, seed treatment can promote control over pathogens when associated with seeds and/or soil. According to Menten and Moraes (2010), seed treatment consists in the application of physical processes or substances (chemical, biological and biochemical) that favor the growth and development of the plant, through the elimination and/or protection of possible pathogens present in the seed, or in the early-stage development in the field. It can havealso beneficial physiological effects on plants, favoring increment of mass, greater resistance to drought periods, and stimulating its self-defense system, among others (CROP LIFE FOUNDATION, 2013).

Among the alternative products that can be used as techniques in disease management, one could mention phosphites (phosphates or phosphorous acid derivatives), despite the registration with foliar fertilizer according to studies has a double action mode in the control of plant diseases, acting directly on the pathogens and also indirectly, inducing defense responses in the plant (DALIO *et al*., 2012), and also in some cases provide nutritional benefits and production increase (NOJOSA, 2002; NOBRE, 2005).

One of the potential methods in disease control is the resistance induction, which is characterized by the activation of the inherent defense mechanisms of the plant. After being submitted to treatment with an inducing substance or organism, it is able to express morphological, physiological and biochemical responses that limit the activity of the pathogen in whose tissues (AGRIOS, 2005).

Therefore, the objective of this work is to evaluate the effects promoted by the treatment of soybean seeds with amino acids and micronutrients-based product (Packseed®), as well as to seek new solutions for the suppression of diseases in the field and also result in increased production and cost reduction.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse at the Federal Technologic University - Parana, Campus Dois Vizinhos. The experiment was conducted in a completely randomized design, in a factorial scheme (2x5), with 5 replicates. The treatments were a product based on amino acids and micronutrients (Packseed®) + conventional seed treatment (insecticide imidacloprid (0.345g of i.a.) + thiodicarb (1.035g of i.a.) fungicide carbedazim (0.75g of i.a.) and polymer (1,5mL) in comparison to the control, where was used only the conventional seed treatment, both in 100 grams of soybean seeds. The second factor was the collection time of the plant material, in 5 different times.

The cultivar used was 5909, sowed in 10 liters' pots, in Red Latosol (Oxisol), at a sowing rate of 10 seeds per pot. The plant material was collected after the emergency (0 hours) and at intervals of 24, 48, 96 and 192 hours. For the collection, 2 cotyledons / healthy and uninjured leaves were taken to perform biochemical analyzes.

For quantification of the enzymatic activity, the biochemical analyzes were performed, beginning with the total proteins quantifications, where after maceration of leaf tissue samples in mortar with 5mL of 0.2 M phosphate buffer solution (pH 7.5), the material was centrifuged (14.000g / 10min at 4°C) and supernatant collected. The BRADFORD (1976) test was used to quantify the total protein content in the samples. Reading was performed in a spectrophotometer, at 595nm, with bovine serum albumin as a standard.

Phenylalanine ammonia-lyase activity (FAL) was determined following the methodology described by Kuhn (2007), by means of the colorimetric quantification of the trans-cyanic acid released from the phenylalanine substrate, and the spectrophotometer reading at 290nm.

For the estimation of chitinase and β-1,3-glucanase activities, were followed the procedures described by Wirth and Wolf (1992),with adjustments, where the samples were macerated in 2,0 mLof 100 mM acetate buffer (pH 5,0), with subsequent centrifugation (20.000 g for 25 min at -4°C). The supernatant was used in the evaluation of enzyme activity.

Chitinase was assessed by the release of soluble "chitin-azure" oligomers from carboxymethylated chitin labeled with bright remazol violet 5R -RBV (Sigma Aldrich®). For the spectrophotometric determination of the β-1,3 glucanase activities in the extracts, bright blue curdlan-remazol substrate (Sigma Aldrich® 4mg.ml-1) was used as substrate.

*Statistical Analysis:*

The data were submitted to analysis of variance by the F test (α0.05) and the differences between the means of the treatments, compared by the Tukey test (α0.05) through the statistical analysis program Assistant Software (SILVA &AZEVEDO, 2009).

## RESULTS AND DISCUSSION

The superior plants have the capacity to respond to chemical stimuli, producing toxic molecules to invading microorganisms. Synthetic compounds are capable to induce a coordinated response of systemic acquired resistance (SAR) in the plant cell which is close (local) or distant (systemic) of the contact points to the substance (SCHNEIDER *et al* ., 1996).

According to the results obtained in the work, the enzyme phenylalanine ammonia-lyase (PAL) was activated at 7 days after emergence (Figure 1), remaining active and stable above the control until 14 days. From the expression of the phenylpropanoid pathway genes, it is induced by SAR-activators and suggests a function for the enzyme PAL, 3-O-methyltransferase of trans cafeoyl-CoA (CCoAOMT) and stilbene synthase (STS) (STS) in the plant's defense system to CCoAOMT, possibly linked to an activation for lignin synthesis, for stiffening and reinforcement of cell walls, and is also a precursor of phytoalexins (NAKAZAWA *et al*., 2001; BUSAM *et al*., 1997). Once the lignified walls can prevent the penetration or development of fungi in plant tissues. (AGRIOS, 2005; PASCHOLATI & LEITE, 1995).

In parallel, preformed chemical substances (glycosinolates and cyanogenic glycosides), toxic or potentially toxic to the microorganism, can be available in the tissues or be modified or synthesized, from the moment it has a contact with an invading pathogen occurs (AHUJA *et al*. 2012).

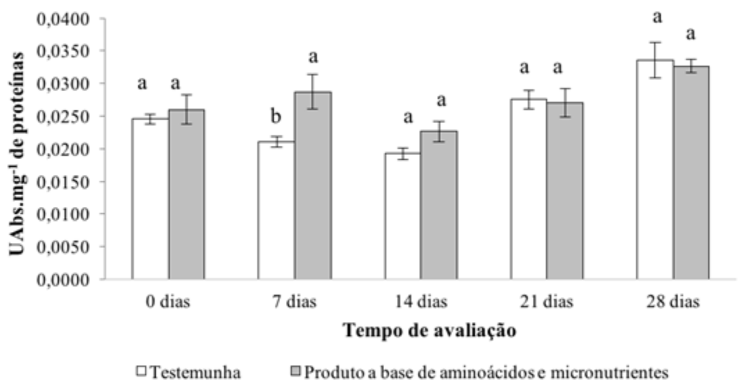


**Fig. I:** Activity of the enzyme phenylalanine ammonia lyase (FAL) in soybean plants, submitted to seeds treatment in different treatments and collected plant material after emergence (0 days) and in intervals of 7, 14, 21 and 28 days. Vertical bars indicate the default error. Different lowercase letters differ from one to another in the treatments, by the Scott-Knott test at 5% probability of error. CV-A = 16,28%, CV-B = 14,65%. UTFPR –Dois Vizinhos, 2017. Source: Author (2017).

In this way, it can be affirmed the product has specificity on action, in other words, it prioritizes only one route of defense (phenylpropanoids) for the production of vegetal defense compounds. However, this hypothesis needs further studies, since the production of the PAL enzyme is regulated during plant growth but is also induced in adjacent cells to the site of infection by various environmental stimuli, such as infection, injury, contamination by heavy metals, light and growth regulators. (MARGIS-PINHEIRO *et al*., 1999)

For the enzyme β-1,3 glucanase (Figure 2), as well as for the chitinase (Figure 3), even with high rates of enzymatic activity at 7 days, there was no significant interaction between the evaluated factors, in other words, no treatment differentiated statistically on the other, at distinct times of plant material collection. Many PRPs manifest direct antifungal activity or other involvement in a cellular defense process. The differential expression of chitinases (a specific group of PRPs that hydrolyzes chitin from the fungal cell wall) is used to prove SAR responses in plants (BUSAM *et al*., 1997).

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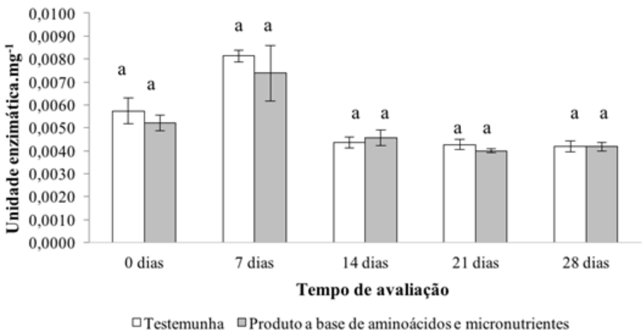


**Fig. II:** Activity of the enzyme β-1,3 glucanase in soybean plants, submitted to seeds treatment in different treatments and collected plant material after emergence (0 days) and at intervals of 7, 14, 21 and 28 days. Vertical bars indicate the default error. Different lowercase letters differ from one to another in the treatments, by the Scott-Knott test at 5% probability of error. CV-A = 16.11%, CV-B = 20.64%. UTFPR –Dois Vizinhos, 2017. Source: Author (2017).

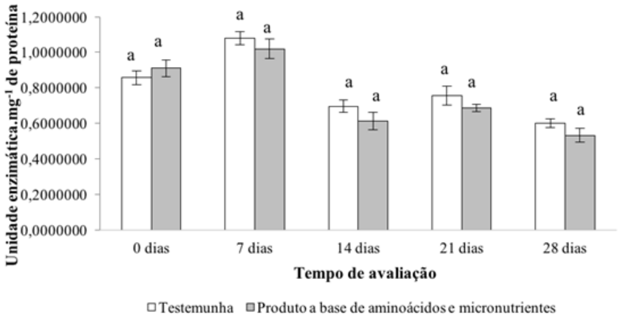


**Fig. III:** Atividade da enzima quitinase em plantas de soja, submetidas ao tratamento de sementes em diferentes tratamentos e coletado material vegetal após a emergência (0 dias) e em intervalos de 7, 14, 21 e 28 dias. Barras na vertical indicam o erro padrão. Letras minúsculas distintas diferem entre si nos tratamentos, pelo teste de Scott-Knott a 5% de probabilidade de erro. CV-A = 10,84%, CV-B = 12,13%.UTFPR - Dois Vizinhos, 2017.Fonte: Autor (2017).

Müller (2015) noted that potassium phosphite in soybean seed treatment has a direct inhibitory action on the mycelial growth of *Fusarium semitectum*, *Pythium sp*. and *Sclerotinia sclerotiorum*. The enzymatic activity tests showed that there is an effect of the phosphites on the induction of resistance, mainly on the activity of the enzyme β-1,3-glucanase, and negatively influences to the activity of enzyme FAL, and other phosphites did not differ significantly from the control.

In the literature, the effectiveness of phosphites is usually related to fungi of the oomycetes group, due to their fungistatic action on this group. Its effects against pathogens are characterized by the inhibition of the oxidative phosphorylation of the oomycetes, retarding their mycelial growth and suppressing their germination and sporulation (LOBATO, *et al*., 2010, SILVA, 2011; DIANESE & BLUM, 2010; PERUCH *ET AL*., 2007). However, its use in relation to other groups of pathogens is not always observed strongly, especially in relation to the parasites classified as biotrophic as *Phakopsora pachyrhizi*.

Gomes *et al*. (2011) obtained positive results when used potassium phosphite in vines to control mildew (*Plasmopara viticola*) but did not show any results for rust (*Phakopsora euvitis*). Oliveira *et al*. (2015) tested the potassium phosphites associated or not with fungicide, did not obtain good results for Asian soybean rust, there was a reduction in disease severity levels in the final stages of the crop when only phosphite and potassium silicate associated. Silva *et al*. (2011) by applying K phosphite in soybean, not only had a significantly reduced rust but also mildew (*Peronospora manshurica*). In a study conducted by Neves and Blum (2014), potassium phosphite reduced the severity of Asian rust with one or two applications and when applied after spraying the fungicides pyraclostrobin + epoxiconazole, methyl thiophanate + flutriafol and tebuconazole.

*Conclusion:*

The results demonstrate the product based on amino acids and micronutrients has the potential to induce resistance by the activation of FAL enzyme. One can affirm the product was able to induce resistance, that bring us back new studies to verify the potential of the product by evaluating its effectiveness in reducing the incidence or severity of the disease, using seed treatment and even sequential spraying of fertilizer based on the same chemical compound, thus evaluating the possible results.

The results obtained are of great importance, since the seed treatment comprises an important practice, which provide the establishment of healthier and more vigorous crops, where it will allow the reduction of the use of fungicides and consequently the cost of production.

*Future Studies:*

To evaluate the resistance induction in common bean from the treatment of seeds based on amino acids and micronutrients (Packseed).

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