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PERCEPTION OF ARBUSCULAR MYCORRHIZAL FUNGUS' SIGNALS BY TOMATO PLANTS (*Solanum lycopersicum* L.) AT INITIAL STAGES OF SYMBIOSIS ESTABLISHMENT

Percepción de señales de los hongos micorrízicos arbusculares por plantas de tomate (*Solanum lycopersicum* L.) en las fases iniciales del establecimiento de la simbiosis

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ABSTRACT. The signal perception established between plants and microorganisms cohabiting with them induce different reactions that range from neutral to defensive. The symbiosis with mycorrhizal arbuscular fungi (AMF) is the most widespread on the planet and the mechanisms used by these fungus to colonize plants as well as the perception of signals emitted are in the focus of research today because the speed which those signals are perceived allow them to compete with other microorganisms present in the rhizosphere for the establishment' niche, taking into account that not all mycorrhizal fungus colonize with the same intensity and that intensity of colonization depends, primarily, on the species- soil fertility relationship. Therefore, this experiment was carried out to compare two of the strains most frequently used from INCA AMF' strain collection in order to check the speed of plants can perceived the signals emitted by these AMF, and it was evaluated using defense enzymes. A swift activation of these enzymes at initial stages of dynamic was observed; but the activation diminishes as soon as recognition achieved. Inoculated plants with *G. cubense* shown faster responses, according the dynamic of activation; this could be according to the criteria of strain recommendation for this specie which has been shown the better agronomic effects for this soil condition.

Key words: mycorrhizae, pectinolytic enzymes, defense mechanisms

RESUMEN. La percepción de las señales que se establecen entre las plantas y los microorganismos que cohabitan con ellas inducen diferentes reacciones que pueden ser desde neutras hasta defensivas. La simbiosis con hongos micorrízicos arbusculares (HMA) es la más extendida en el planeta y los mecanismos por los cuales colonizan a la planta, así como la percepción de las señales que emiten se encuentran en el foco de investigación de la actualidad, dado que la rapidez con que sean percibidas las señales les permitirá competir con otros microorganismos presentes en la rizósfera por el nicho de establecimiento, teniendo en cuenta que no todos colonizan con la misma intensidad y que esto depende en primer término de la relación especie-fertilidad del suelo. Por ello se realizó este experimento de comparación con dos de las cepas más utilizadas del cepario del Instituto Nacional de Ciencias Agrícolas (INCA) para comprobar la rapidez de la percepción de las señales de estos HMA por las plantas y se evaluó a través de dos enzimas relacionadas con la defensa. Se observó una rápida inducción de las actividades de estas enzimas al inicio de la dinámica, que disminuyó rápidamente en la medida que se inició el reconocimiento entre los simbiontes. Las plantas inoculadas con *G. cubense* respondieron más rápido, según pudo observarse en la dinámicas de activación y esto podría relacionarse con el hecho de ser la cepa recomendada para esta condición de suelo por sus efectos agronómicos demostrados.

Palabras clave: mycorrhizae, enzimas pectinolíticas, mecanismos de defensa

INTRODUCTION

Plants are living organisms that get in touch with other organisms in their environment. As plants cannot see, hearing or walking, they emit signals that enable them perceiving the changes in the environment and surviving. Plants produce a great variety of

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chemicals, namely sugars, amino acids, fatty acids, growth regulators and secondary metabolytes some of which are used to the communicate with their environment (1).

Among the interactions taking place in the rhizosphere, the following can be mentioned: root-root, root-microorganisms and root-insects. Many of these interactions have a neutral effect on plants. However, at the rhizosphere too, those interactions established with mutualists or the ones that are beneficial, can also be found (2).

As a result, the plant-microorganism poses a considerable potential for biotechnological exploitation. A good example would be to be able to manipulate the signals taking place at the rhizosphere, in which plants use roots to communicate and interact with other microorganisms (1).

Microorganisms in turn, can affect the growth and development of the tolerance of plants positively or negatively as well as changing the dynamics of the nutrients, the susceptibility to diseases, tolerance to heavy metals and besides they can help plants in the degradation of xenobiotics (3). As a result, the interaction plant-microorganism poses a considerable potential for biotechnological exploitation. A good example would be to be able to manipulate the signals taking place in the rhizosphere, in which plants use roots to communicate and interact with other microorganisms in (1).

Among the most ancient symbiotic associations of the plants arbuscular mycorrhizae can be found that are established with the fungi phylum Glomeromycota. This symbiosis, formed by more than 80 % of earth plants, is considered the most extended symbiosis in the planet and its development results in the formation of sub-cell structures inside the plant known like arbusculos that are the main site of nutrient exchange between the plant and its associated pair (4). AMF connect to the plant through an hyphal network that can exceed 100 meters of hyphae per cubic centimeter of soil and it is especialized in the uptake of nutrients and water (5). In return, plants supplement fungi with carbohydrates from the photosynthesis. Twenty percent of the photosynthesis products (close to 5 billion tons of coal per year) is estimated to be consumed by the mycorrhizal fungus. Therefore, fungi significantly contribute to the cycling of phosphate and carbon and influence on the productivity of earth ecosystems (6).

The bidirectional control exerted by both symbionts implies important changes in the primary and secondary metabolism and the regulation of the defensive mechanisms of the plant (7) and have an important impact on its physiology by altering the capabilities of the plant to confront biotic and abiotic stresses (8, 9, 10).

It is known that not all AMF turn out to have equal efficiency under different edaphic environments and there is a specific relationship between their efficiency, in agronomical terms, fertility and the pH of the soil (11, 12). It could be thought that due to these reasons they determine the perception of the signals emitted by these fungi, and also their efficiency in terms of competition for the colonization niche, as well as for the fastness they colonize plants and therefore start to pass from the biotic stress stage to the symbiont's[A].

In spite of that, studies on the perception of signals indicating these fungi induce in the plant still find in initial stages for the fact of their obligatory biotrophism (1, 13), hence this research sets the objective of evaluating the changes in the activities related to defense induced by two AMF in tomato plants at the initial stage of establishing symbiosis.

MATERIALS AND METHODS

Plant material

The tomato variety Amalia (*Solanum lycopersicum* L.) produced by the Department of Genetics and Breeding of the National Institute of Agricultural Sciences (INCA) was used as a study model because the induction of defensive systems in late stages of colonization is known.

Fungal material

Isolates from the AMF fungi *Glomus mosseae* (Nicolson & Schenck), INCAM-2; *G. cubense* (Y. Rodr.& Dalpé), INCAM-4; from the isolate collection of the Mycorrhizae Laboratory of INCA were used. Species were preserved in a substrate developed for these purposes by the Mycorrhizae Laboratory of INCA (Patent registration No. 2264) at 4 ºC. AMF inocula used in the experiments had an average title of 50 spores g^{-1} of substrate, certified by the Mycorrhizae Laboratory of INCA.

Experimental conditions

The experiment was conducted with rootballs and a heat-sterilized substrate at 121ºC for 1 h during three alternate days, making up a mixture of Red Ferralitic Lixiviated Typical Soil (14) and earthworm humus at the ratio of 3:1 (p/p). The agrochemical characteristics of the substrate are shown in Table I.

Plants developed under controlled temperature conditions (23 ºC ± 2 ºC), relative humidity (80-85 %) and natural photoperiod (14 hours of light-10 hours of darkness). Trials were done under glass house conditions in areas of the National Center for Animal Health (CENSA), San José de las Lajas, Mayabeque, Cuba.

[A] Pérez, E. *Hongos micorrízicos arbusculares (HMA) para la bioprotección de patógenos en el cultivo del tomate (Solanum Lycopersicum L.)* [Tesis de Doctorado], Universidad de La Habana, La Habana, Cuba, 2010.

Tabla I. Características agroquímicas del sustrato

Location	Substrate	K^+	Ca^{2+}	Mg^{2+} (cmol kg^{-1})	P (ppm)	Organic matter (%)	pH (al H_2O)
San José de las Lajas, La Habana	Red ferralitic lixiviated typical soil: earthworm humus (3:1)	0,6	18,9	6,0	160	6,9	7,3

Chemical determinations: pH at H_2O, Potentiometer; Organic Matter (MO), Walkley Black; Phosphorus (P), Oniani; Cations, Ca^{2+}, Mg^{2+} and K^+, Maslova's Method

Dynamics of temporal and local induction of PR 2 (β 1,3 glucanase) and PR 3 (quitinase), in mycorrhized plants with promision isolates

In order to evaluate the induction of PR2 and PR3 in the early hours of the mycorrhization process, seeds were sown at the substrate shown in table 1 and were kept under the conditions previously described. In the day 21 (after germination) plants were inoculated through their roots with the selected mycorrhizal fungi using a liquid formulation of spore suspension (10^5 spores mL^{-1}), in an osmotically-protected solution (National Patente's Request, OCPI 2004-0272). Root samples were taken at 1, 2, 4, 8, 12, 18, 24, and 48 hours, after applying the suspension.

Root samples were macerated in liquid nitrogen and homogenized at the rate of 1:2 ($g.mL^{-1}$), with extraction buffer solution (Sodium acetate 0,1 M, pH 5,2; that contained 5 g of polyvinylpirrolidone and 0,05 g of β-mercaptoethanol, in 100 mL of extraction solution). The homogenate was agitated on a screen for 45 minutes, on an ice bath. Later on it was filtered and centrifuged at 14 000 x G, at 4 °C for 25 minutes, on a refrigerated centrifuge (Beckman, model J2-21). The supernatant was stored at -20 °C till its use, for determinations of the enzymatic activity by the methodologies shown in Table II; protein concentration was determined according to Bradford (1976).

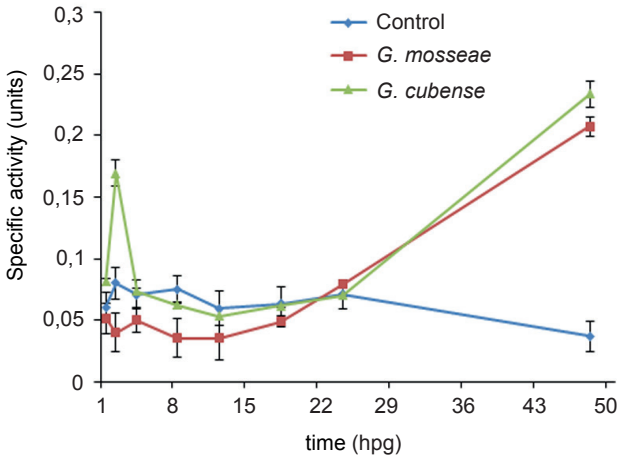
Experimental design and statistical data analysis

Experiments were conducted following a totally random design with three repetitions, they were made in two different moments.

The confidence interval of the means at 95 % of probability was calculated based on the number of repetitions and the reproducibility of the data.

RESULTS AND DISCUSSION

Figure 1 shows the activation dynamics found at local level, in roots, for the enzyme β 1, 3 glucanase hours after being inoculated with AMF under study. It is seen that induction is fast, since enzymatic activity levels were detected, that in the case of *G. cubense* exceeded non-inoculated plants since hour 1 till hour 4. The isolate *G. mosseae*, though with values lower than control's, also showed activity levels since hour 1. In the late hours of this dynamics (43 hours) there was a significantly higher increase in treated plants as compared to the non-inoculated control.



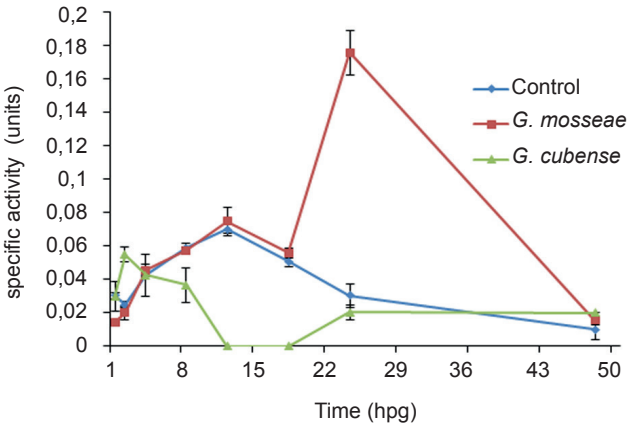
Bars represent confidence intervals of the mean for (p≤0,05 %) (n=6)

Figure 1. Induction dynamics of the activity β 1-3 glucanase (PR 2) in tomato roots inoculated with *G. mosseae* and *G. cubense* hours after the mycorrhizal induction

Table II. Determinations

Determination	Quantification method	Substrate	λ nm	Units (referred to as U.A.E)
β-1,3-glucanases (PR2)	Dangrois et al. (1992)	Laminarina	450	µKat mg^{-1} protein
Chitinase (PR3)	Boller et al. (1983)	Coloidal Chitin	585	pKat mg^{-1} protein

Figure 2 shows the activation dynamics of the chitinase enzyme in roots of inoculated plants hours after the induction of mycorrhization. Inoculated plants with *G. cubense* showed activation levels at the beginning of the dymamics that went down at 12 hours: these plants show a new induction at 17 hours that remains till the end of the dynamics. Plants treated with *G. mosseae* showed a similar behavior to control plants, which shows a low activity at the beginning and a maximum activity at 12 hours. Control plants reduced the activity from this moment on till the end of the dynamics, not hapenning the same with mycorrhized plants with *G. mosseae* that showed a maximum activation at 24 hours and then reduced in time.



Bars represent confidence intervals of the mean for p ≤0,05 % (n=6)

Figure 2. Induction dynamics of the chitinase activity (PR 3) in tomato roots mycorrhized with *G. mosseae* and *G. cubense,* hours after of inducing mycorrhization

In general, the PRs induction at local level was quick which suggests that the perception of signals established between isolates and tomato plants was effective.

The induction of defensive mechanisms by these arbuscular mycorrhize forming fungi is depressed as a consequence of the molecular recognition among symbionts, which brings about a suppression of the molecular events that permit the defensive action by the plant; it coincides with the results of other authors (15). Such depression is mainly caused by the secretion of AMF fungi of an efective protein that promotes symbiotic biotrophy thus acting over the defensive genes of plants. This fact promotes the colonization of these AMF fungi in the root system from the perception of fast signals that caused induction levels of PR proteins that tended to decrease after confronting symbionts; it should be the cause of the detected reduced activities al it is also indicative that as soon as the plants and AMF start interacting, the dynamics in time of defensive mechanisms should go down, it is considered that they pass from a stage of biotic stress to symbionts which can be predicted by the quick establishment of signals between both parts.

The results of the early induction of β 1, 3 glucanase, as well as the detection of basal levels in non-mycorrhized plants coincide with those reported. That found activation levels of the enzyme since the early hours of colonization. The basal levels of the activity for both enzymes could be associated to cell differenciation processes. In the case of mycorrhized plants, these levels also, can be associated to the hyphal growth in plant roots[A].

The presence of a maximum activity in inoculated plants with *G. cubense* at the beginning of the dynamics of β 1,3 glucanase, indicates that the AMF presence on the root induces the activation of this enzyme, which, in addition to act on the previously mentioned cell differentiation processes, also takes part in the degradation of cell walls of the fungi because of the presence of β 1,3 glucanes in them (16). It allows the recognition between the plant and mycorrhizal fungi that is more evident in the isolate *G. cubense*. Moreover, it has been reported that signal perception by plants produces morphological changes in the host cell that starts to accomodate for the colonization by forming a pre-penetrating appratus that involves the activation of enzymes favoring hyphal growth of the fungi in the roots (17).

Likewise, the present chitinase in the plants, due to its role in the defense responses, generates the production of elicitors that induce weaker responses in mycorrhized plants than in those facing pathogenic microorganisms (13).

The elevation of the enzymatic activities at the beginning of the dynamics performed, followed by a fast decrease that reaches up to the basal levels of activity, supposes a fast interaction and recognition between the plants and the AMF. It is confirmed than the recognition between the plant and the AMF fungi makes the defensive response stops on the plant (13, 15) and that symbiont's fast recognition rapidly suppresses the defensive patterns of it.

The fact that induction and the recognition understood as the activation and the reduction of the enzymatic activities allows pointing at isolate *G. cubense* as the one that was the quickest perceived by the plant which could be related to the fact that this isolate is the recommended one for this condition of fertility and pH of the soil for its agronomic effects.

BIBLIOGRAPHY

1. López-Ráez, J.A.; Bouwmeester, H. y Pozo, M.J. ‘‘Communication in the Rhizosphere, a Target for Pest Management’’ [en línea], ed. Lichtfouse, E., *Agroecology and Strategies for Climate Change*, edit. Springer Netherlands, 2012, (ser. Sustainable Agriculture Reviews, no. ser. 8), pp. 109-133, ISBN 978-94-007-1904-0, [Consultado: 14 de marzo de 2015], Disponible en: <http://link.springer.com/chapter/10.1007/978-94-007-1905-7_5>.
2. Raaijmakers, J.M.; Paulitz, T.C.; Steinberg, C.; Alabouvette, C. y Moënne-Loccoz, Y. “The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms’’, *Plant and Soil*, vol. 321, no. 1-2, 23 de febrero de 2008, pp. 341-361, ISSN 0032-079X, 1573-5036, DOI 10.1007 s11104-008-9568-6.
3. Morgan, J.A.W.; Bending, G.D. y White, P.J. “Biological costs and benefits to plant–microbe interactions in the rhizosphere’’, *Journal of Experimental Botany*, vol. 56, no. 417, 2005, pp. 1729–1739, ISSN 1460-2431.
4. Parniske, M. ‘‘Arbuscular mycorrhiza: the mother of plant root endosymbioses’’, *Nature Reviews Microbiology*, vol. 6, no. 10, 2008, pp. 763–775, ISSN 1740-1534.
5. Verbruggen, E.; van der Heijden, M.G.A.; Rillig, M.C. y Kiers, E.T. ‘‘Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success’’, *New Phytologist*, vol. 197, no. 4, 1 de marzo de 2013, pp. 1104-1109, ISSN 1469-8137, DOI 10.1111/j.1469-8137.2012.04348.x.
6. Dodd, I.C. y Ruiz-Lozano, J.M. “Microbial enhancement of crop resource use efficiency’’, *Current Opinion in Biotechnology*, vol. 23, no. 2, abril de 2012, (ser. Food biotechnology - Plant biotechnology), pp. 236-242, ISSN 0958-1669, DOI 10.1016/j.copbio.2011.09.005.
7. Kiers, E.T.; Duhamel, M.; Beesetty, Y.; Mensah, J.A.; Franken, O.; Verbruggen, E.; Fellbaum, C.R.; Kowalchuk, G.A.; Hart, M.M.; Bago, A. y others “Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis’’, *Science*, vol. 333, no. 6044, 2011, pp. 880–882, ISSN 1095-920, DOI 10.1126/science.1208473.
8. Miransari, M. “Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress’’, *Plant Biology*, vol. 12, no. 4, 1 de julio de 2010, pp. 563-569, ISSN 1438-8677, DOI 10.1111/j.1438-8677.2009.00308.x.
9. Smith, S.E.; Facelli, E.; Pope, S. y Smith, F.A. “Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas’’, *Plant and Soil*, vol. 326, no. 1-2, 19 de mayo de 2009, pp. 3-20, ISSN 0032-079X, 1573-5036, DOI 10.1007/s11104-009-9981-5.
10. Campos-Soriano, L.; García-Martínez, J. y Segundo, B.S. “The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defence-related genes in rice leaves and confers resistance to pathogen infection’’, *Molecular Plant Pathology*, vol. 13, no. 6, 1 de agosto de 2012, pp. 579-592, ISSN 1364-3703, DOI 10.1111/j.1364-3703.2011.00773.x.
11. Herrera-Peraza, R.A.; Hamel, C.; Fernández, F.; Ferrer, R.L. y Furrazola, E. “Soil–strain compatibility: the key to effective use of arbuscular mycorrhizal inoculants?’’, *Mycorrhiza*, vol. 21, no. 3, 16 de junio de 2010, pp. 183-193, ISSN 0940-6360, 1432-1890, DOI 10.1007/s00572-010-0322-6.
12. Fester, T. y Sawers, R. “Progress and Challenges in Agricultural Applications of Arbuscular Mycorrhizal Fungi’’, *Critical Reviews in Plant Sciences*, vol. 30, no. 5, 1 de septiembre de 2011, pp. 459-470, ISSN 0735-2689, DOI 10.1080/07352689.2011.605741.
13. Jung, S.C.; Martinez-Medina, A.; Lopez-Raez, J.A. y Pozo, M.J. ‘‘Mycorrhiza-Induced Resistance and Priming of Plant Defenses’’, *Journal of Chemical Ecology*, vol. 38, no. 6, 24 de mayo de 2012, pp. 651-664, ISSN 0098-0331, 1573-1561, DOI 10.1007/s10886-012-0134-6.
14. Hernández, A.; Pérez, J.; Bosch, D. y Castro, N. *Clasificación de los suelos de Cuba 2015*, edit. Ediciones INCA, Mayabeque, Cuba, 2015, p. 93, ISBN 978-959-7023-77-7.
15. Kloppholz, S.; Kuhn, H. y Requena, N. ‘‘A Secreted Fungal Effector of Glomus intraradices Promotes Symbiotic Biotrophy’’, *Current Biology*, vol. 21, no. 14, 26 de julio de 2011, pp. 1204-1209, ISSN 0960-9822, DOI 10.1016/j.cub.2011.06.044.
16. Gianinazzi-Pearson, V. ‘‘Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis.’’, *The Plant Cell*, vol. 8, no. 10, 1996, p. 1871, ISSN 1532-298X.
17. Bonfante, P. y Requena, N. ‘‘Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis’’, *Current Opinion in Plant Biology*, vol. 14, no. 4, agosto de 2011, (ser. Biotic interactions), pp. 451-457, ISSN 1369-5266, DOI 10.1016/j.pbi.2011.03.014.

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