

Mechanism of action of *Trichoderma harzianum* as controlling agent of *Fusarium subglutinans*

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Recibido 25 de marzo de 2013; Aceptado 20 de junio 2013

Resumen

Los expertos estiman que del 10 al 30% de los granos cosechados, dependiendo de la tecnología de almacenamiento y del clima, se pierde debido al deterioro producido por los hongos; por ejemplo, los niveles actuales de la producción y comercialización de piña (*Ananas comosus* L.), no logran abastecer las necesidades del país debido a daños provocados por *Fusarium moliniforme* var. *subglutinans*, generando así, la necesidad de utilizar alternativas naturales. Los hongos antagonistas son importantes para el control biológico de los fitopatógenos. En este sentido, las especies del género *Trichoderma harzianum* se destacan entre las más utilizadas para el biocontrol de patógenos fúngicos del suelo. Estas especies presentan mecanismos de combate, dentro de los cuales se encuentran especialmente: competencia por el sustrato y nutrientes, micoparasitismo y antibiosis; aun cuando cabe destacar que mientras mayor sea la probabilidad de que un aislamiento de *Trichoderma*, manifieste varios modos de acción; más eficiente y duradero será el control sobre el patógeno, aspectos que no poseen los plaguicidas químicos. El objetivo del presente artículo es reconocer los mecanismos de acción del *T. harzianum* como agente para el control del hongo *F. subglutinans*.

Palabras claves: *Fusarium subglutinans*, *Trichoderma harzianum*, competencia, micoparasitismo, antibiosis.

Abstract

Experts estimate that 10 to 30% of harvested grain, depending on storage technology and climate, is lost due to deterioration caused by fungi, for example, current levels of production and marketing of pineapple (*Ananas comosus* L.), fail to meet the needs of the country due to damage caused by *Fusarium moliniforme* var. *subglutinans*, thus generating the need for natural alternatives. Antagonistic fungi are important for the biological control of plant pathogens. In this sense, the genus *Trichoderma harzianum* rank among the most widely used for biocontrol of soil fungal pathogens. These species have mechanisms to fight, within which are especially: competition for substrate and nutrients, antibiosis mycoparasitism and, even when digging out that the greater the probability that an isolate of *Trichoderma*, indicates several modes of action, more be efficient and durable control of the pathogen, both of which have no chemical pesticides. The aim of this article is to recognize the mechanism of action of *T. harzianum* as an agent for control of the fungus *F. subglutinans*.

Key Words: *Fusarium subglutinans*, *Trichoderma harzianum*, competition, mycoparasitism, antibiosis.

Introducción

Biological control is defined as any category, class or practice through which the survival or activity of a pathogen is decreased through the mediation of any other body, regardless of the man, with a decline in the incidence of disease. The use of microorganisms to control plant diseases and other microbiological problems that limit productivity has received increased attention in recent times (Lindow and Wilson 1997). Most field conditions have been manipulated with chemical pesticides. These have been applied to the soil, seeds, foliage and fruit, but they have presented negative consequences on human health and the environment (Kookana and Simpson 2000) for chemical applications, the residual (O'Keefe and Farrell 2000) and the development of resistance to

pesticides (Ho 2000), produced the search replacement pesticides with biological agents that can be used in controlling (Inbar and Chet 1997). The researchers aimed primarily work to understand the ecology and epidemiology of pathogens, as well as beneficial microorganisms.

The control of pathogen plants by microorganisms is well documented and is not only of academic interest, but commercial (Wilson and Backman 1998). There are few commercial products on the world market so for some diseases, represent a viable alternative to use. Furthermore, advances in biotechnology supported on molecular biology, biological control make alternative control for potential pathogens without harming the environment.

The favorable conditions for the spread of *Fusarium* belonging to the class hyphomycetes,

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Order and Family Moniliales Tuberculaceae (G. Agrios 1995), are mainly long and frequent periods of Tang wet during flowering (40-60 hours), high humidity relative (80 %), temperatures of 24-26 °C, nutritional deficiencies and plenty of shade (PROCAFE 1997). *Trichoderma* species produces three types of propagules: hyphae and conidia calmidoplastos (Diaz 1994).

The symptoms of the disease appear unilaterally, accompanied by partial yellowing of leaves (one half and the other green chlorotic normal) and the bending of shoots on the side of the diseased plant; turn dwarfism observed reduction in plant growth (Arbeláez 2000). Fungal hyphae penetrate directly or through the wound in the epidermis of the root pass and endodermis cortex and enter the xylem vessels invading them when ripe (Ochoa 1996). The developments of the fungus in the plant vascular tissue affect water transport. The lack of water induces closed stomata of the leaves, the leaves wither and the plant dies (Agrios 1988).

FAO estimates that annually at least 25 % of the world's crops are affected by mycotoxins with substantial impact of *Fusarium* species (Charmley et al. 1994), also in Argentina, Formento and Ramos (1993) found an average loss of 40, 6% in 1991 in the as epiphyte of 1991 and according Cultivar, planting date and yield potential it has have been established losses up to 50 % (Annone et al. 1994). In Nayarit, the proportion of affected orchards was 100,100, 50 and 50 % for the municipalities of Tepic, San Blas, Santiago de Compostela, respectively (Vazquez and Perez 1996)

This type of *Fusarium*, is an impotent economic problem, according to Rodriguez (1993), the cost of fighting pests and diseases of pineapple fruit (*Ananas comosus* L) without *Fusarium subglutinans* is U.S. \$ 145/ha, while their presence, added a cost of \$ U.S. 215/ha, representing a total of \$ U.S. 360/ha.

Despite this, it is considered that the species most widely studied and applied as biological control for this disease (Obregon 2002) belonging to the genus *Trichoderma*, due to its effective control reproductive ability, ecological plasticity, stimulating effect on crops and acting as an inducer of systemic plant resistance to various pathogens (Cervantes 2007, Harman 2004). *Trichoderma* species produce three types of propagules: hyphae, and conidia calmidoplastos.

Trichoderma most have no sexual period simply produce asexual spores. However, at a few lines of

Trichoderma it is known sexual period between lines but not used in biological control. The sexual stage, when it occurs, is among the Ascomycetes genus *Hypocrea*. Its taxonomy is traditionally based on morphological differences, initially in asexual sporulation bodies, but now they are using more molecular studies. Because of these recent studies, the taxon has gone from nine to at least thirty- three species (Yedidia et al. 1999).

Trichoderma harzianum is a fungus that is also used as a fungicide. It is used in foliar, seed and soil treatment for the control of various fungal diseases. Some commercial products produced by this fungus are effective in the control of *Botrytis* and *Fusarium*. Also used in the manufacture of enzymes.

Given the above, the objective of this article is to make a compilation of the main mechanisms of action of *Trichoderma harzianum* as a control agent of *Fusarium subglutinans*.

Trichoderma harzianum biology

T. Harzianum colonies grow and mature rapidly after five days of incubation in growth media and potato dextrose agar (PDA) at 25 ° C. The species of this genus generally prefer an acidic pH of 4.5-5 (Pandey and Tewari 1990), The minimum moisture content for vegetative growth is 92 % and for sporulation is 93 to 95 %.

The life cycle begins when the organism grows and branches as a typical fungal hyphae measuring 5-10µ diameter, asexual sporulation also occurs when spores of 3-5µ diameter are released in a large number. Chlamyospore are also interspersed (Seaby 1996) soft green from 12,5 to 10 microns in size (Hoog 2000).

Biological control with antagonistic microorganisms began to be investigated steadily from the 80s (Hernández et al. 2007), so that the *Trichoderma harzianum* has been used to combat postharvest fungi as *Botrytis cinerea* on grapes, partially controlling spot disease (Latorre et al. 1997). It has also been used against *Botrytis cinerea* and *Penicillium expansum* on apples, protecting the fruit for two months in situ (Lifshits et al 1986, Spotts et al 1986).

Action Mechanisms

Within the action of *Trichoderma* biocontrol is, competition for space and nutrients, mycoparasitism and antibiosis.

As a first step, the COMPETITION is defined as

unequal behavior of two or more bodies with the same requirement (substrates, nutrients). This type of antagonism is favored by the characteristics of the biological control agent as ecological plasticity, growth rate primarily as chlamydospores (Hjeljord and Tronsmo 1998, Pérez 2004), speed of development, and external factors such as soil type, pH, temperature and humidity (Ahmad and Baker 1987).

Nutrient competition, can occur for nitrogen nonstructural carbohydrates (sugars and polysaccharides such as starch, cellulose, chitin, laminarin, and pectin, among others) (Stefanova et al 1999) and micro elements. It stands, which in a situation of complete fertilization or over some of the components of fertilizers including organic matter, such antagonism is not very effective, for example, iron in a soluble state (10⁻⁸ M at neutral pH) is one of the factors that set stiff competition (Tronsmo Hjeljord 1998).

Mycoparasitism: is another type of parasitic pathogen antagonism where a broad spectrum of species of fungi such as *Rhizoctonia*, *Sclerotium*, *Sclerotinia*, *Helminthosporium*, *Fusarium*, *Armillaria*, *Colletotrichum*, *Verticillium*, *Venturia*, *Endothia*, *Pythium*, *Phytophthora*, *Rhizopus*, *Diaporthe*, *Fusicladium* (Ahmed et al 2000), it is usually implicated extracellular enzymes such as chitinases, cellulases corresponding to the composition and structure of the cell walls of fungi parasitized (Ulloa 1996).

Trichoderma species mycoparasitism during chemotropically process grow towards the host, hyphae adhere to it, wound on them frequently and sometimes penetrate. Degradation of the cell walls of the host is observed in the late stages of the process parasitic (Carsolio et al 1999), which leads to almost total fitopathogen weakening.

This process is explained by Chet and Benhamou (1998), it has been separated into four stages, within which it is recognized:

Chemotrophic growth: the positive chemotropism direct growth toward a chemical stimulus (Chet and Inbar 1994).

Recognition: Performed through lectin-carbohydrate interactions. (Chet et al 1998).

Adhesion and curl: occurs when the acknowledgment response is positive, *Trichoderma*

hyphae adhere to host mediated enzymatic processes (Perez 2004). Hyphae adhesion occurs through association of a sugar antagonist wall with a lectin present in the wall of the pathogen (Martinez et al 1994).

Lytic activity: this stage production occurs extracellular lytic enzymes, mainly chitinases, glucanases and proteases, which degrade the cell walls of the host and allow the penetration of antagonist hyphae (Haram et al 1996).

Trichoderma excreted metabolites (cellulases, glucanases, lipases, proteases and chitinases) which facilitate the insertion of hyphae for nutrient uptake interior of the pathogen (Eveleigh et al 1986), ending with the loss of cytoplasmic contents of the host cell. The remaining cytoplasm is mainly surrounding invading hyphae, showing signs of disintegration (Diaz 1994).

Elad et al (1983), recommends *Trichoderma* isolates based on its ability to produce D-1,3- glucanase and chitinase in the presence of chitin and glucan, respectively, some Authors also reported hyphal different interactions as parasitism (strap 1997). For example Bernal et al (2004) found penetrating hyphae curl and in hyphae of *Trichoderma F. oxysporum* f. sp. *Cubense*, *Pythium* sp., *R. solani*.

Antibiosis lastly: is the inhibition of pathogen development by metabolized products (Benitez et al 1998) and small molecules toxic, volatile and lytic enzymes (Baker and Griffin, 1995), which operate structural polymers, such as chitin and β -1-3- glucans of the cell wall in most pathogenic fungi, producing an adverse effect on development and differentiation (Goldman et al 1994).

Given the above, it is said that the greater the amount of metabolic products, the antagonistic power increases (Pezet et al 1999), additionally some authors believe that this mechanism should not be the main, due to the risk of emergence of the antibiotic-resistant pathogens (Vero and Mondino 1999), such as *Agrobacterium* strains resistant *tumesfaciens* Agrosin 84 (Campbell 1989).

Dennis and Webster (1971), performed work regarding the role of antibiotics produced by fungi of the genus *Trichoderma* on pathogen plants. Germain and Oliver later reported that *T. harzianum* produces many antibiotics such as: trichodermina, suzukacilina, alamethicin,

dermadina, trichotecenos and trichorzianina (Martinez 1998).

Martinez *et al* (2008) observed to evaluate 59 isolates of *Trichoderma*, substrate competition, mycoparasitism and antibiosis. In nearly all isolates they identified at least one type of interaction decreasing hyphal well defined by a side control and decreases the possibility that arises in the pathogen resistance to the antagonist (Vero 1999).G

Final considerations

The department of Caquetá with an average temperature of 28 °C and an annual rainfall of over 4.000 mm, represents favorable conditions for the development of fungal pathogens in soil, for example the presence of *Fusarium Subglutinans* in pineapple crops, currently occupy an area approximately 313 hectares with a production of 1.446 tons.

Consequently, it is feasible high biocontrol potential of the *Trichoderma harzianum* for the variety of mechanisms of action, for the more modes of action are present in isolation, the greater the effectiveness of the same in the control and thus less damage which can cause the crop. To which adds facilities incubation colonies growing and mature rapidly after five days of incubation in growth media and potato dextrose agar (PDA).

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