Chapter

A Review Report on the Mechanism of *Trichoderma* spp. as Biological Control Agent of the Basal Stem Rot (BSR) Disease of *Elaeis guineensis*

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Abstract

Trichoderma spp. have been the most common fungi applied as biological control agents (BCA) as an effort to combat a wide range of plant diseases. Its uses have recorded good success rate in controlling major plant diseases. Knowledge on the mechanisms employed by *Trichoderma* spp. could be further studied to improve its ability as an efficient biocontrol agent. The *Trichoderma* ability to curb plant diseases were mainly based on the activation of single or multiple control mechanisms. It is known that the *Trichoderma*-based biocontrol mechanisms mainly rely on mycoparasitism, production of antibiotic and/or hydrolytic enzymes, competition for nutrients, as well as induced plant resistance; numerous secondary metabolites produced by *Trichoderma* species could directly inhibit the growth of several plant pathogens. These mechanisms may act directly or indirectly against the targeted plant pathogen. This chapter reviews the recent updates on published research findings on mechanisms used by *Trichoderma* as biological control of plant diseases particularly on basal stem rot disease of oil palm caused by *Ganoderma* spp.

Keywords: antibiosis, competition, induced resistance, mycoparasitism, secondary metabolite

1. Introduction

1.1 Biological control of oil palm basal stem rot disease caused by *Ganoderma boninense*

Many promising biological antagonists, mainly from *Trichoderma*, *Aspergillus*, *Penicillium*, *Pseudomonas*, and *Bacillus*, have been reported as effective antagonists against *Ganoderma boninense* in coconut [1] and oil palm [2–4]. In 1990, [5] evaluated the incorporation of *Trichoderma* spp. grown on dried palm oil mill effluent into planting holes as a prophylactic measure. Later, [6] reported delays in infection in the field following treatment with *Trichoderma*, but eventually, the disease incidence was similar to untreated controls. Thus, the possible explanation for this could be due to a low natural occurrence of *Trichoderma*

spp. in the soil [7]. In Malaysia, certain *Trichoderma* strains such as *Trichoderma virens* and *Trichoderma harzianum* have shown good biocontrol ability against *G. boninense* in nurseries [8]. Besides that, in Indonesia, a biofungicide consisting of *Trichoderma koningii* was reported to reduce BSR in decomposing oil palm residues in the field [9].

Biological control of BSR disease in oil palm can be effectively achieved through the utilization of an effective strain of *Trichoderma* spp. The strain must not only have the potential mechanisms for biological control such as antibiosis and mycoparasitism but also a strong competitive ability to displace the causative fungus *G. boninense* so as to reduce the pathogen's opportunity for root colonization. It must be able to favorably compete and adopt well within the environment in which it will operate and be able to rapidly colonize and proliferate on the existing and newly formed roots immediately after its application [10].

2. Mechanisms of *Trichoderma* species

Trichoderma spp. employ several antagonistic mechanisms against plant pathogens. These include antibiosis, mycoparasitism, competition for nutrients and space, promotion of plant growth, induced plant defense mechanisms, and modification of environmental conditions [11].

2.1 Mycoparasitism

The potential of *Trichoderma* spp. to parasitize, suppress, or even kill other plant pathogenic fungi has been recognized as an important mechanism for its success as a biological control [12]. Mycoparasitism is a direct mechanism for biological control that works by parasitizing, detecting, growing, and colonizing pathogen [11, 13]. The ability to mycoparasitize other fungi has been widely used for the biological control of agricultural pests (mainly against pathogenic fungi and parasitic nematodes). Some species of *Trichoderma* such as *T. asperellum*, *T. atroviride*, *T. virens*, and *T. harzianum* are widely used as biological control agents of plant pathogens [11]. It is able to directly kill pathogens and other plant-associated fungi, with a wider host range in diverse ecologies [14]. This is done via the use of many mycoparasitic strategies [15, 16]. These mycoparasitic abilities appear to be very complex, involving the detection of plant pathogen through chemotropism; lysis of the pathogen's cell wall (the key to mycoparasitism) [17]; pathogen's hyphal penetration by appresorial formation; production of cell wall-degrading enzymes (CWDEs) and peptaibols, mediated by heterotrimeric G-proteins and mitogen-activated protein (MAP) kinases [11]; and parasitizing pathogen's cell wall contents [12]. Degradation of pathogen's cell wall during mycoparasitism is mediated by a set of hydrolytic enzymes including β-(1,6)-glucanases, chitinases, and proteases. Several members from each of these classes have been shown to be involved in mycoparasitism and/or to be induced under mycoparasitism-related growth conditions [18]. Genome analysis enabled the assessment of cell wall-degrading enzymes encoded in the genomes of *Trichoderma* spp. and unraveled even more complex enzymatic degradation machinery for fungal cell walls than previously anticipated [19].

Considerable research work has been done to identify and understand the enzymes induced by *Trichoderma* to recognize host pathogen [20]. The degradation of a pathogen's cell wall is an important aspect of mycoparasitism and biological control of plant diseases. *Trichoderma* also produces secondary metabolites (volatile and nonvolatile) [21]. With regard to the production of secondary metabolites, two

Trichoderma species—*T. virens* and *T. reesei*—are the highest producers of secondary metabolites [22].

Three important *Trichoderma* species—*T. virens*, *T. atroviride*, and *T. harzianum*—have been identified with the highest production of chitinolytic enzymes compared to other fungi known to have similar biological control abilities [21]. Most of the secondary metabolites' coding gene clusters are exclusively specific to certain *Trichoderma* spp., while some are found in all three species [21]. Studies on the responsible signal transduction pathways of *T. atroviride* during mycoparasitism have led to the isolation of key constituents of MAP and cAMP kinase signaling pathways, such as the α-subunits of G-proteins (G-α) that control antibiotic production, extracellular enzyme, and coiling around host hypha [23].

2.2 Promoting plant growth

Microbial organisms colonize a plant's root system and at the same time play a beneficial role in biological control, protecting the plant from soil-borne pathogens as well stimulating plant growth [13]. These beneficial relationships between plants and microbes often occur in the rhizosphere, improving plant growth or helping the plant overcome biotic or abiotic stresses [24]. *Trichoderma* spp. proliferate in the rhizosphere, establishing a symbiotic association, thus improving plant nutrition and growth in a natural way [25]. It is able to colonize roots, improving plant nutrition, growth, and development as well as enhancing plant resistance to abiotic stresses. Increasing plant growth by using biological control agents is usually attributed to an indirect effect associated with control of plant pathogens. It was also reported that the application of *T. harzianum* to cucumber or tomato seedlings increased the concentration of trace and essential elements such as Fe, Zn, Cu, Mn, Mg, Ca, N, P, K, and Na both in the shoots and roots [26]. This is due to its ability to produce many phytohormones, siderophores, and phosphate-solubilizing enzymes [27]. Phytohormones stimulate root growth, thus increasing the absorptive surface of plant roots. These phytohormones include cytokinins, indole-3-acetic acid, and gibberellins [28]. Growth promotion of plant antimicrobial compounds of *Trichoderma* has been demonstrated [29]. Harzianopyridone, 6PP, trichocereus A-D, koninginins, cyclonerodiol, harzianolide, and harzianic acid (HA) are examples of isolated compounds that promote plant growth in a concentration-dependent manner [30]. A novel secondary metabolite—cerinolactone—has been isolated and characterized from *Trichoderma cerinums* and was able to positively alter the growth of tomato seedlings 3 days after treatment [30]. Similarly, HA and iso-harzianic acid found in *T. harzianum* metabolites were also found to promote plant growth, through the strong binding of iron [30]. It was also shown that *T. virens* and *T. atroviride* produce certain types of indole-3-acetic acid-related indoles (IAA-related indoles). The production of IAA-related indoles in *Trichoderma* liquid cultures was stimulated by the addition of L-tryptophan. This observation proposed that the production of IAA-related indoles could be one of the mechanisms employed by *Trichoderma* to promote plant growth and an increase in the number of secondary roots, leading to a higher biomass in *Arabidopsis* [31]. On the other hand, [32] proposed that degradation of IAA by *Trichoderma* leads to a reduction of detrimental effects of IAA on root elongation that could cause reduced ethylene (ET) production, by decreasing its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), and/or ACC deaminase activity present in *Trichoderma*. Recently, it was shown that *T. asperellum* has high 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) activity when grown with ACC as the sole nitrogen source. The ACCD-encoding gene Tas-acdS was upregulated when ACC was added to the artificial growth medium. Silencing of Tas-acdS showed decreased ability of silenced

transformants to promote root elongation of canola plants [33]. These mechanisms could be responsible for the plant growth-promoting activity of *Trichoderma* [31, 33]. The application of *Trichoderma* spp. results in significant vegetative growth on a wide range of crop plants [31, 34]. Interaction between *Trichoderma s*pp. and the plant triggers enhanced immunity against plant diseases, thus improving plant health [35]. The ability to promote plant growth may be an important characteristic; however, this is not found in every *Trichoderma* species [13, 36]. Plant growth enhancement is evidenced by the increase in productivity, nutrient uptake, biomass, resistance to stress, and improvement of plant health [37]. *Trichoderma* isolates from the rhizosphere of the mangrove *Avicennia marina* solubilize P from the insoluble $Ca_3(PO_4)^2$ and correlate with an increase of the extracellular phytase activity—an acidic phosphatase and extracellular activity of phytase were induced only in the presence of $Ca_3(PO_4)^2$ [38]. In addition, the application of *Trichoderma* spp. in consortium was reported to enhance the physical strength and durability of the plant's cell wall toward cell wall-degrading plant pathogenic fungi [39–42]. It is likely that improvement in root growth was the effect of one or more mechanisms, and this may include an increase in soil nutrient solubilization, increase in the rate of carbohydrate photosynthetic activities and carbohydrate metabolism, plant growth regulatory effect, and increased rooting depth, thus increasing resistance to drought conditions [43]. *Trichoderma* spp. are more effective in colonizing and enhancing plant growth, if there are enriched inorganic soil substrates such as bioorganic fertilizers [44].

2.3 Induced resistance in plants

Apart from *Trichoderma*'s capacity to attack plant pathogens and inhibit its growth, many reports have shown the induction of systemic and local resistances against a wide range of pathogens [25, 45]. Both the inductions of systemic and local resistances in plants are the result of complex interactions between different elicitors released by microbes and plant receptors, which induces plant cells' physiological and biochemical changes. However, these processes are possible when the systemic acquired resistance (SAR) triggers a resistance in the entire foliage and enhances production of the defense signal molecule, salicylic acid (SA), for further signaling [46]. In addition, *Trichoderma* spp. were reported to induce the synthesis of regulatory proteins in plants especially under certain disease stress, where these regulatory proteins detect microbe effectors and activate the plant's defense systems [47]. Induction of systemic resistance by *Trichoderma* spp. has been scarcely studied, as compared to the response induced in plants by rhizobacteria, presumably because the research on *Trichoderma* focused on understanding the factors involved in antibiosis and mycoparasitism. Its ability to induce systemic and local resistances has been reported on both monocots and dicots [12, 31, 43, 45]. It is important to know that induced resistance varies from plant to plant due to the influence of environmental factors, the pathogens involved, and the symbionts' relationship in the rhizosphere [34, 48]. Plant's resistances are induced via the expression of pathogenesis-related (PR) genes mediated by SA and are known as the SAR, which is also triggered by biotrophic and/or hemibiotrophic pathogens [25].

The secondary metabolites 6-pentyl-α-pyrone and harzionalide produced by *T. atroviride* and *T. harzianum* induced systemic defense response in oilseed rape and tomato seedlings against *Leptosphaeria maculans* and *B. cinerea*. In addition, upregulation of PR genes was also found in plants treated with these secondary metabolites [49]. Expression analysis of SA-related genes in *Trichoderma* spp.-treated plants showed a long-lasting upregulation, possibly activating a priming mechanism in the plant. Inoculation of *Trichoderma*-treated plants with *B. cinerea*

led to an enhanced expression of JA-related genes, triggering systemic resistance to the pathogen in a plant genotype-dependent mode [36]. Peptaibols also induce systemic resistance and defense responses in plants—*T. virens* produces peptaibols of 14 and 18 amino acids, with several isoforms each that induce systemic resistance [50]. In plant root colonization, *Trichoderma* spp. deal with a plant's defense system by synthesizing antimicrobial compounds such as phytoalexins. Its interactions with plants during the early stages of root colonization might stimulate the activation of cell detoxification and plant protection mechanisms [51].

2.4 Antibiosis

Trichoderma spp. are rich and important sources of secondary metabolites (SMs) used for biological control of plant diseases [12]. It was reported that antibiosis occurs during the interactions between a host plant, pathogens, and *Trichoderma* spp. that resulted in the production of antibiotics and low-molecular-weight compounds by *Trichoderma* to inhibit the growth of phytopathogenic fungi [52]. Fungal secondary metabolites also play important roles in interactions with plants [53]. Antimicrobial activities could be the result of several secondary metabolites such as peptaibols, terpenes, polyketides, gliotoxin, and gliovirin produced by fungi [49]. Other metabolites include tricholin, harzianic acid, viridian, gliosoprnins, heptelidic acid, 6-pentyl-α-pyrone, and massoilactone [54]. Secondary metabolites are classified into two major classes—useful and toxic compounds polyketide synthases and non-ribosomal peptide synthases [55] or volatile and nonvolatile secondary metabolites which deter colonization by competing with pathogenic fungi in ecological niches. Secondary metabolites are known for its ability to synthesize peptaibols. Peptaibols are from a family of peptides with antibiotic functions, characterized by C-terminal alcohol residues, short-chain-length amino acids (<20 residues), and high levels of nonstandard amino acids [21]. Peptaibols consist of 2-amino-isobutyric acid and other non-proteinogenic amino acids, produced as secondary metabolites with antibiotic activities against pathogenic fungi and bacteria. It has been discovered that peptaibols produced by *T. pseudokoningii* can induce programmed cell death in plant fungal pathogens, causing apoptotic deaths of the pathogens [56]. These are natural products biosynthesized by many fungi and work together with cell wall-degrading enzymes to inhibit or completely prevent the growth of pathogenic fungi and/or elicit development of induced plant resistance against pathogens [21]. Research has shown that these compounds demonstrated strong positive effects on plant growth and resistance in plants to abiotic and biotic stresses [57]. The production of antimicrobial compounds strongly depends on the availability of exogenous nutrients such as root exudates, leakage of nutrients on leaf surface, and/or organic nutrients in the soil, as well as environmental conditions [58]. Due to the advancement in analytical studies, many secondary metabolites from *Trichoderma* spp. have been isolated and identified. Over 120 *Trichoderma* secondary metabolites have been identified and reported by [59]. There are over 1000 *Trichoderma* peptaibol sequences known to date—some involved in biocontrol. This great potential of *Trichoderma* spp. to produce several types of secondary metabolites is reflected in the genomes of the three important species (http://genome.jgi-psf.org/) which amounted to 262 and 349 for *T. reesei* and *T. atroviride*, respectively. Most of the secondary metabolite genes present in *T. reesei* are also found in *T. atroviride* and *T. virens* [19]. Peptaibols—peptides containing a-aminoisobutyric acid (Aib) and a C-terminal 1,2-amino alcohol—are produced largely by members of *Trichoderma* [60]. Peptaibols have unusual amino acid content that resulted from non-ribosomal biosynthesis. Several functional enzymes known as peptide synthetases assemble these molecules via the multiple

carrier thiotemplate mechanism from a remarkable range of precursors, which can be N-methylated, acylated, or reduced. Peptaibols show interesting biological and physiochemical properties that include the formation of pores in bilayer lipid membranes and antifungal, antibacterial, and rarely antiviral activities and elicit plant resistance [61]. These secondary metabolites induce certain reaction in plants when applied to the leaves or injected into roots or plant tissues. It also stimulates the biocontrol potential of *Trichoderma* by activating mycoparasitic gene expression which elicits defense mechanisms against plant pathogens [62].

Some exert antimicrobial effects at high doses, but others act as microbe-associated molecular patterns (MAMPs), while auxin-like compounds act at low concentrations. For example, even 1 ppm of 6-pentyl-α-pyrone, harzianolide, or harzianopyridone can activate a plant's defense system and regulate growth in plants (e.g., tomato, canola, and pea) [49]. Many elicitors released by *Trichoderma* that activate plant defense systems can be classified into several groups such as proteins or peptides [31], enzymes (cellulase, chitinase, glucanase, protease, or xylanases) [63, 64], and fatty acids, lipids, and its derivatives such as glycosphingolipids [12].

2.5 Competition

This is a classical mechanism of biological control of plant pathogens [65]. Competition among microorganisms occurs only when resources such as soil nutrients and space are limited. In this situation, antagonistic microbes produce secondary metabolites capable of inhibiting or slowing growth and other activities of pathogenic fungi, thus conferring ecological advantages over its competitors. Antagonistic microbes utilize available resources for growth, leaving pathogens with insufficient nutrients for its growth and consequently starved [66]. These antagonists favorably compete for iron causing the suppression of *Fusarium* spp. [67]. Iron is an essential mineral nutrient for many microbes. Iron in an aerobic condition (i.e., with oxygen and neutral pH) exists as Fe^{3+} in immobilized forms rather than as oxyhydroxides or hydroxides, thereby making it unavailable for microbes [68]. *Trichoderma* has a strong potential to mobilize and utilize soil nutrients, making it more efficient and competitive than many other soil microbes (fungi and bacteria). This process could be related to the production of inorganic acids, namely, citric, gluconic, and fumaric acids which decrease soil pH and increase the solubilization of phosphate and micronutrients (iron and manganese) [49]. *Trichoderma* secretes siderophores and is able to grow in conditions that are poor in iron by using residual immobilized Fe. Most fungi including *Trichoderma* produce various forms of siderophores, which help the fungi to overcome adverse soil conditions [68]. Siderophore production can be beneficial to the plant and microbes for two reasons: (1) siderophore production by antagonist fungi cansuppress the growth of plant pathogens by depriving it of an iron source and (2) siderophores can help in solubilizing iron that was unavailable to the plant [12]. Most of the siderophores isolated so far belong to the hydroxamate class and can be divided into three structural families: caprogens, ferrichromes, and fusarinines. Fungi typically produce more siderophores than other microbes [12]. Specifically, *T. harzianum* produces the highest amount of siderophores but does not have any unique compounds, while *T. reesei* can biosynthesize cis-fusarinine, a major siderophore. The variety of siderophore production in *Trichoderma* spp. is due to the modification of the non-ribosomal peptide synthetase (NRPS) products rather than diverse NRPS-encoding genes [69]. The ability of tetramic acid from *Trichoderma* spp. to bind with good affinity to Fe^{3+} explains the mechanism of iron solubilization that significantly changes nutrient levels in the soil for other microbes and the host

plant. Siderophores produced by *Trichoderma* spp. have other effects and functions including virulence enhancement of pathogens, storage of intracellular iron, and suppression of microbe growth during competition with *Trichoderma* spp. [68].

Reduced nutrient concentrations lead to reduced conidial germination, slowed germ tube growth of a pathogen, reduced number of infection sites, and the extent of plant disease development [70]. *Trichoderma* has the ability to alleviate a wide range of abiotic and physiological stresses and also enhances plant nutrient uptake and increases nitrogen use efficiency. *Trichoderma* root colonization delayed the effects of drought-induced changes in stomatal conductance, green fluorescence emissions, and net photosynthesis resulting in an improved plant water status [71]. This may potentially be more important for plant disease control because *Trichoderma* deprives pathogens of available nutrients for growth and development, thus rendering it ineffective to cause any disease. Some *Trichoderma* species even demonstrated a potential to improve photosynthetic and respiratory activities of plants, resulting in its ability to reproduce plant gene expression, probably through the activation of a limited number of general plant pathways [25].

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