Lignification on Potatoes by Application of Trichoderma viride

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Abstract. Leaf blight disease caused by pathogenic fungus Phytophthora infestans is the major disease in potato that can reduce its production up to 100%. The use of biological agent Trichoderma viride as an inducing potato resistance against leaf blight disease has been considered potential method. The purpose of this study was to evaluate the use of biological agent Trichoderma viride in inducing potato plant resistance against late blight by Phytophthora infestans. Research on induced resistance was conducted in a greenhouse with polybag using Completely Randomized Design with 6 treatments and 6 replications. The parameters observed were the growth and the leaf blight intensity. Induced resistance by using Trichoderma viride against Phytophthora infestans increased in total phenolic compounds in the third and fourth weeks as well as lignification. Observation of lignification on the fifth week showed that lignification occurred in leaf xylem of plants tissue. Trichoderma viride application could improve the systemic resistance of potato plants.

1. Introduction

Potato (Solanum tuberosum) is the fourth most important foodstuff in the world after wheat, corn, and rice [1] and it contains the second highest carbohydrate after cereal [2]. In Indonesia, potato is one of the most important horticultural commodities, and it is considered to be alternative food ingredients supporting food diversification programs. However, the main problem in cultivating potato in highlands is leaf blight disease caused by pathogen Phytophthora infestans in addition to the limitations of quality seeds. Leaf blight disease is very damaging and difficult to control, as P. infestans is a pathogenic fungus having various levels of pathogenicity that occur because this fungus is heterotalic [3]. Consequently, loss due to leaf blight disease can reach 100% [4].

An alternative method known to be very potential to control the disease biologically is antagonistic fungi to pathogenic P. infestans by inducing resistance host plants against potential pathogenic attacks. The antagonist fungus Trichoderma spp [5]. It suppresses the pathogenic fungi by being hyperparasitic, mycoparasitic, and inducing host plant resistance to potential pathogenic attacks [6].

Controlling potato leaf blight disease by induced resistance of plants with antagonistic fungi Trichoderma spp. is part of biological control because it utilizes nonpathogenic microorganisms as an induced plant resistance. As a result of the resistance affected by inoculation of biological agents, disease symptom decreases and biochemical factors of the host plant changes leading to the plant to be resistant to disease-causing pathogens. The induced resistance of plant by biological agents operates by activating the genetic potential of host plant resistance.

In general, as any pathogens infect a plant, the defense mechanism within the plant will be activated to be a protection. Plants defend themselves in two ways; (i) the presence of structural properties that function as physical barriers and inhibit pathogens from entering as well as spreading within plants, and (ii) biochemical responses in the form of chemical reactions taking place in plant cells and tissues so that pathogens can die or stunt their growth.

Phenolic compounds formed in response to injured plants are generally shown by increased synthesis of cinnamic acid derivatives, called chlorogenic acid and caffeic acid [7]. In general, chlorogenic acid and
caffeic acid that function to resist the attack of microorganisms are precursors of the protective layer that is synthesized near the damage and precursors in the biosynthesis of lignin [8]. The resistance of potato varieties to the pathogenic fungal attack of *Phytopthora infestans* in Indonesia has not been much studied, including its resistance mechanism. Based on this, it is necessary to study the mechanism of resistance of potato plants to the application of *Trichoderma viride* antagonistic fungi.

Given the explanation, this aim of this study was to determine the effect of *Trichoderma viride* antagonistic fungi application on potato plant resistance by identifying indicator of potato plant resistance such increased total phenol and lignin content in the leaf tissue of the potato plants.

2. Materials and Methods

The study was conducted in a potato screen house at the Vegetable Crops Research Institute (BALITSA), located in Cikole Village, Lembang District, Bandung City, West Java Province with an altitude of ± 1200 meters above sea level from March to July 2013. The potato seeds variety used Granola (G2), the one susceptible to leaf blight, obtained from BALITSA Potato Seed Center. The medium for planting, consisted of a mixture of soil and compost/manure with a ratio of 3:1, was filled into a 50 cm in diameter polybag. Prior to this, the medium was physically sterilized using soil sterilizer (steamer). Potato tubers were then planted in the polybags (one tuber in one polybag), and were maintained for approximately 4 months.

The study used a completely randomized design (CRD) consisting of 6 treatments and 6 replications. The experiment was conducted in the greenhouse using Completely Randomized Design (CRD) method with 6 treatments, each of which was repeated 8 times. The six treatments were P1: Application of *T. viride* antagonists 2 weeks before planting potato seedlings (MST) and *P. infestans* pathogenic fungal infections; P2: Application of *T. viride* antagonists 1 week after planting potatoes (MSD) and infection pathogenic fungus *P. infestans*; (P3): Application of *T. viride* antagonists 2 mst and 1 mst as well as *P. infestans* pathogenic fungal infections; (P3): Application of synthetic fungicides made from active mankozeb (Dithane M-45) and fungal pathogen infections *P. infestans*; (P5): Without *T. viride* inoculation of potato plants but infected by pathogen *P. infestans* (positive control), and (P6): Potato plants that were not applied to *T. viride* or were not infected with the pathogen *P. infestans* (negative control) (P6).

The *T. viride* antagonist fungal isolate was rejuvenated on a PDA medium for 8 days at room temperature (25°C), then the conidial density was calculated to be $10^8$ conidia/mL. The pathogenic isolates of *P. infestans* were also grown on V8 gelatin juice medium for about 9 days in an incubator at 18°C. Furthermore, grown sporangia were harvested by scraping the surface of the V8 gelatin juice medium with a glass rod. The sporangia density was calculated to be $10^3$ sporangia/mL. Conidium and sporangia density were calculated using a haemocytometer. A total of 250 mL of *T. viride* conidia suspension solution with the above density was poured into the soil where potato seedlings were grown following each treatment. Furthermore, at the age of 30-days, a total of 300 mL of *P. infestans* zoospore suspension with zoospore density of $10^6$ was also inoculated on leaves on potato plants that were 30 days old except in the positive control treatment. The relative humidity was maintained at a minimum of 90% and the relative air temperature was set at 20°C. Increased induction of potato plant resistance by antagonistic fungi *Trichoderma viride* can be seen through the process of lignification in the leaf tissue organs of the potato plant.

3. Results and Discussion

Defense from cell walls against pathogenic infections, accumulation or occurrence of lignin other than in roots which can occur in leaves or on potato tubers [8]. The mechanism of lignification on the stems and leaves of potato plants was observed in several periods of in planta observation, which was carried out on the leaves of potato plants infected with leaf blight at the age of 0, 45, 60 days after planting either not inoculated with antagonistic fungi or those inoculated with *Trichoderma* antagonistic fungi *sp*. Before reaching the infection process, a pathogen had to be able to overcome barrier protecting the plant. Mostly, pathogenic fungi infiltrated a plant through wounds or naturally exposed plant parts, such as stomata or actively penetrating plant surface layers. This barrier structure included a cell wall which was usually composed by layers of lignin, cuticles, and was related to the pectin layer of the cell wall that covers
the entire aerial surface of the plant. During the second cell wall thickening process, plant cell walls and intercellular spaces were filled with phenolic lignin polymers. Lignification occurred enzymatically through dehydrogenation and was followed by condensation of radical coumaryl, coniferyl, and sinapyl alcohol. Chemical analysis indicated differences in the composition of the induced material compared to lignin in healthy tissue.

Potato plants treated with the *T. viride* antagonist fungus (P1, P2 and P3), a dark red color appeared on the cross section of the leaf xylem cell wall. This indicated that the level of lignin cell wall xylem of potato plants applied by antagonistic fungi was higher than the level of lignin in plants without being applied by the *T. viride* antagonist fungi (P1, P2 and P3) (Figure 1). In the three treatments (P1, P2 and P3), xylem cells appeared in the transverse slices of the potato leaf cells which were more numerous and thicker in the cell wall. Whereas, plants without antagonistic fungi application have smaller number (the color of xylem cell walls tends to be brighter than the color of xylem cell walls in plants treated with antagonistic fungi) (Figure 1).

In plant leaf cells applied with antagonistic fungi, in the treatment of P1, P2 and P3, more lignin was formed within the cell wall as a form of structural defense against pathogenic fungal attack. This could be identified from the transverse staining of potato plant leaves having xylem cells color of dark red and tended to be thicker. In addition, Figure 1 shows the number of xylem cells were more than the number of xylem cells from leaves of the potato plants that were not applied by antagonistic fungi (Figure 1). The response mechanism of the potato plants associated with enhancing resistance was structural changes of the potato plant with the formation of antifungal compounds, the formation of defense proteins, the formation of the enzyme khitinase, lignification and hypersensitive reactions. The results of the cross section staining of potato leaves showed that there was more lignification in potato plants applied by antagonistic fungi than plants without application.

The potato plant was induced by, in this case, antagonist fungus *Trichoderma viride* that had been given to the plants before transmission or infection by pathogenic fungi. Therefore, the impact resistance was included in post inflectional defense (defense after infection). Control of soil borne pathogens such as pathogenic fungus *Phytophthora infestans* could be done after antagonistic fungi colonized the roots. The scavenging is done by pre-inoculation with the scavenging microorganisms called elicitors or inducers in the form of non-pathogenic bodies, avirulent pathogens or in the form of chemicals [9] [10]. Microscopic view of cross section of leaf xylem cells in the treatment of P1, P2, P3, P4, P5 and P6 Potato Plants aged 60 days

![Figure 1. Cross section of xylem cells (B) of potato plant leaves and lignification (A) in P1 treatments](image)
Figure 2. Cross section of xylem cells (B) of potato plant leaves and lignification (A) in P2 treatments

Figure 3. Cross section of xylem cells (B) of potato plant leaves and lignification (A) in P3 treatments

Figure 4. Cross section of xylem cells (B) of potato plant leaves and lignification (A) in P4 treatments
One parameter for the mechanism of plant resistance to pathogenic infection is an increase in phenol compounds [11]. An increase in phenol in cotton seedlings inoculated with *Rhizoctonia solani* and the highest phenol concentration occurred on the 6th day after inoculation [12]. The increase in phenolic compounds such as cinnamic acid, cyclic acid, caffeic acid, ferulic acid, sinapat acid and lignin-forming phenol monomers (synapyl alcohol, coniferil, and p-comaril) are then followed by the formation of peroxidase enzymes that can be used as an indication of lignification [13].

Chemical changes in the potato plant that thicken the xylem cell wall inhibited the development of pathogenic fungi. It was seen as a structural barrier to pathogens entering the potato plant tissue. The phenol compound could also be directly toxic to attack by pathogens so that it functioned as phytoalexin by forming conjugated phenol, lignification or cell wall suberization. Induced resistance in plants resulted in a reduction in disease symptoms, due to changes in biochemical factors in plants that were local or systemic.

The increase phenol compounds caused the increased enzyme lyase. This increase in enzymes caused resistance induction due to the association of Trichoderma antagonist fungi and potato plant roots. The antagonistic fungi respond to plant resistance because it can suppress pathogens. Potatoes which were applied with the *T. viride* antagonist turned out to increase the induction of plant resistance due to lignification and an increase in phenol content. Fungal pathogen infection of *P. infestans* can also reduce
phenol content in tissues [14]. The accumulation of phenols in plant cell walls is effectively reported to inhibit the development of pathogenic mycelia and cell wall degrading enzymes [15].

4. Conclusions
Application of antagonistic fungus of Trichoderma viride had an effect to increase the induction of plant resistance due to lignification and an increase in phenol content on potato plant.

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