Induction of Resistance Using *Trichoderma* spp. and *Penicillium* sp. against Banded Leaf and Sheath Blight (BLSB) Caused by *Rhizoctonia solani* in Maize

Amarilisa Kusuma, Ani Widiastuti, and Achmadi Priyatmojo

Abstract Banded leaf and sheath blight (BLSB), caused by *Rhizoctonia solani*, is an important disease in maize. In this study, *Trichoderma* spp. and *Penicillium* sp. were used to control the disease. Experiments were conducted with five treatments: control (no treatment) (R_0); *R. solani* inoculation (R_1); *Trichoderma* spp. and *R. solani* inoculation (R_2); *Penicillium* sp. and *R. solani* inoculation (R_3); and combined *Trichoderma* spp., *Penicillium* sp., and *R. solani* inoculation (R_4). The results showed that the heights of maize plants treated with R_3, R_2, or R_4 did not differ significantly in comparison with R_0 treatment but did differ significantly in comparison with R_1 treatment. The numbers of leaves in maize plants treated with R_4, R_2, or R_3 differed significantly in comparison with R_0 and R_1 treatment. The stem girths of maize plants treated with R_2, R_3, or R_4 did not differ significantly in comparison with R_0 treatment, but a significant difference was observed in comparison with R_1 treatment. Peroxidase enzyme activity with R_0, R_2, R_3, or R_4 treatment was increased at 4 days and 8 days after inoculation; on the other hand, enzyme activity with R_1 treatment was increased only at 4 days after inoculation and was then decreased at 8 days after inoculation. The intensity of disease ratings with treatments R_0, R_1, R_2, R_3, and R_4 were about 2%, 28%, 10%, 9%, and 5%, respectively.

Keywords Induced resistance • Maize • *Penicillium* sp. • *Rhizoctonia solani* • *Trichoderma* spp.

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1 Introduction

Maize (*Zea mays* L.) is a source of carbohydrate [1] and food for humans and livestock, as well as a source of industrial materials for products such as starches and biofuels [2]. Sweetcorn (*Zea mays* var. *saccharata* Sturt) is a commodity that can be cultivated intensively [3]. This study focused on banded leaf and sheath blight (BLSB), caused by *Rhizoctonia solani*, which is an important disease in maize. *R. solani* is a plant pathogenic fungi and is important because it has a wide range of hosts [4]. Worldwide, both the quality and quantity of maize have been increasingly affected by BLSB caused by *R. solani* [2]. The grain yield loss caused by this disease has increased from 11% to 40%, and even to 100% in some cultivars in some warm and highly humid regions, where the conditions are favorable for the pathogen [5, 6].

In this study, *Trichoderma* spp. and *Penicillium* sp. were used to control the disease. *Trichoderma* species, as biological control agents, antagonize a range of soilborne phytopathogenic organisms and can suppress pathogens through competition for space and nutrients [7], parasitism, and antibiosis [8, 9]. During the interaction of *Trichoderma* with the plant, different classes of metabolites may act as elicitor as plant resistance inducer compounds [10–12]. Species of *Penicillium* are fundamentally cosmopolitan and ubiquitous, and many of them have been thoroughly studied with regard to their ability to produce mycotoxins that can contaminate food [13–15]. With reference to *R. solani*, so far, antagonistic activity has been observed only for a few *Penicillium* species [16–20]; in some cases, it has been reported in relation to the production of toxic metabolites [17, 19, 20].

Like many plant species, maize uses a diverse array of defenses to minimize losses during attack by a pathogen. In addition to preexisting physical and chemical barriers, a variety of defense mechanisms are activated upon attack by a pathogen [21]. Biochemical changes in many plant–pathogen interactions are accompanied by rapid increases in phenolic compounds and related enzymes, often termed a *hypersensitive response* [22]. Some studies of biochemical changes during pathogenesis have revealed that certain defense biomolecules such as phenols and sugars, as well as enzymes such as peroxidase and polyphenols, are formed to increase in levels, thereby altering resistance against the pathogen [23]. Such changes can be attributed to a variety of mechanisms of defense as exhibited by the host during pathogenesis [24].

2 Methods

Experiments were conducted using five treatments, including a control (no treatment) (R_0); *R. solani* inoculation (R_1); *Trichoderma* spp. and *R. solani* inoculation (R_2); *Penicillium* sp. and *R. solani* inoculation (R_3); and combined *Trichoderma* spp., *Penicillium* sp., and *R. solani* inoculation (R_4). Disease intensity was assessed using the method described by Vimla and Mukherjee [25].

Extracts were prepared by weighing 200 mg of the sample, homogenized in 10.0 mL of ice-cold phosphate buffer (0.1 M, pH = 6.5) in a prechilled mortar–pestle. The homogenate was centrifuged at 2 °C at 10,000 rpm for 15 min in a refrigerated centrifuge. The clear supernatant obtained was collected and separated into two 5 mL portions. One 5 mL portion was kept on ice under refrigerated conditions and used for estimation of the activities of peroxidase and polyphenol oxidase. The other 5 mL portion was kept at room temperature and used for estimating the contents of total phenols [26].

Peroxidase activity was estimated by the protocol of Manoranjankar and Mishra (1976) [27]. Here again, the first 5 mL portion of the crude extract preparation kept under 0–40 °C was used, and 3.0 mL of the assay mixture was used for peroxidase activity estimation, comprising 2.3 mL of 0.1 M phosphate buffer (pH 6.5), 0.5 mL of guaicol substrate, 0.1 mL of the enzyme extract, and finally 0.1 mL of H_2O_2 (5%) to start the reaction. The assay components were quickly mixed and transferred to a spectrophotometer cuvette for recording of changes in absorbance at 15 s intervals for a maximum time of 3 min. Each observation was recorded for peroxidase activity against a substrate blank. Enzyme activity was calculated on the basis of changes in absorbance per minute per milliliter of the enzyme in the reaction mixture. As the substrate got transformed into the product, a colorless to dark brown oxidation product was formed by 3 min time.

3 Results and Discussion

Figure 1 shows that the tallest maize plants were seen with the R_3 treatment, monitored every week after planting. Figure 2 shows that the numbers of maize plant leaves with the R_2, R_3, and R_4 treatments, monitored every week after planting, did not differ significantly. Figure 3 shows that the stem girths of maize plants with the R_2, R_3, and R_4 treatments, monitored every week after planting, did not differ significantly. The results were related to the abilities of *Trichoderma* and *Penicillium* as plant growth–promoting fungi (PGPF).

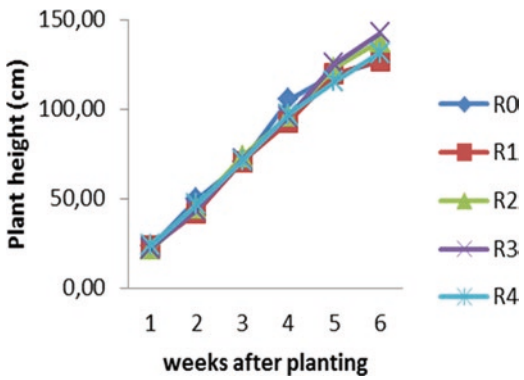

Fig. 1 Heights of maize plants with treatments monitored every week after planting

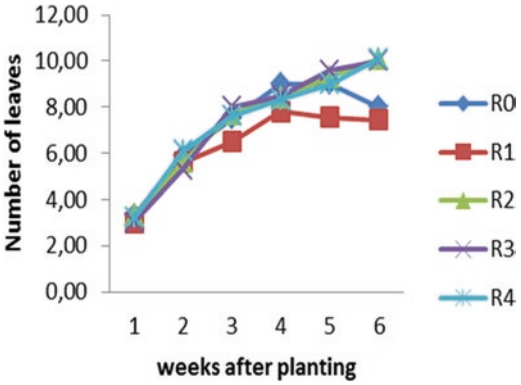

Fig. 2 Numbers of maize plant leaves with treatments monitored every week after planting

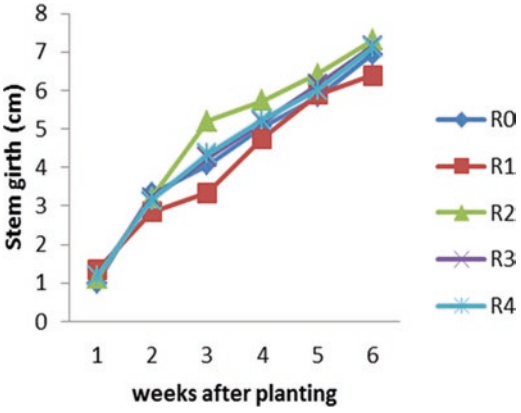

Fig. 3 Stem girths of maize plants with treatments monitored every week after planting

Some well-documented ISR-inducing fungi are mycorrhiza, *Trichoderma* sp., *Fusarium* sp., *Penicillium* sp., *Pythium* sp., and *Phoma* sp. Most of them fall into the category of plant growth–promoting fungi (PGPF), widely distributed in rhizosphere soils [28, 29]. Therefore, some *Trichoderma* strains are more suitable for biological control as biopesticides and others are more suitable for stimulating crop growth and nutrient uptake, acting as biostimulants [30–36]. When grown at the rhizosphere or on the root surface, *Trichoderma* is expected to face frequent interactions with other plant microorganisms, such as arbuscular mycorrhizal (AM) fungi. Indeed, such interactions have been investigated in the past, with contrasting results. In some cases, inoculation with both fungi resulted in positive synergistic effects on the plants or in the inhibition of plant growth [31, 32, 37, 38].

The intensity of disease ratings with treatments R_0, R_1, R_2, R_3, and R_4 were about 2%, 28%, 10%, 9%, and 5%, respectively (Fig. 4). The symptoms started appearing as large, discolored areas alternating with irregular dark bands. The disease developed on leaves and sheaths and spread to the ears. Characteristic symptoms include concentric bands and rings on infected leaves and sheaths that are discolored—brown or gray in color. Typically, the disease develops on the first and second leaf sheaths above the ground and eventually spreads to the ear, causing ear rot (Fig. 5).

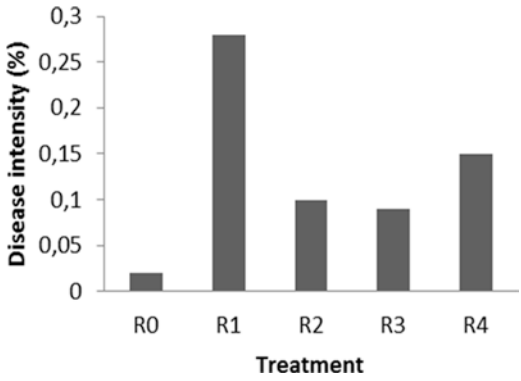

Fig. 4 Disease intensity (%) of banded leaf sheath blight caused by *Rhizoctonia solani* 6 weeks after pathogen inoculation

Fig. 5 Symptoms of banded leaf sheath blight caused by *Rhizoctonia solani* 6 weeks after pathogen inoculation

In this study, estimation of peroxidase activity was done 1 day before treatment and 4 days and 8 days after treatment. Peroxidase was increased at 4 days and 8 days after the R_0, R_2, R_3, and R_4 treatments but was decreased at 8 days after the R_0 treatment (Fig. 6). Peroxidase activity was found to be increased in plants infested with *Fusarium* (28%) and *Alternaria* (27%) [39]. This showed that *Trichoderma* has the ability to increase peroxidase activity after pathogen inoculation. In a previous study, Yedidia et al. (1999) [40] provided evidence that *T. asperellum* may

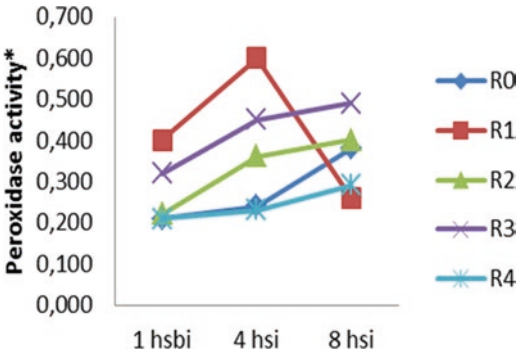

Fig. 6 Estimations of peroxidase activity monitored 1 day before treatment and 4 days and 8 days after treatment (*1 U = change in 0.01 absorbance per min per mg of protein)

induce a transient systemic increase in the activities of peroxidase and chitinase and in production of phytoalexins.

Ethylene is a volatile product of the fungus *Penicillium*. Stimulative effects of ethylene on increases in peroxidase and polyphenol oxidase were first reported by Stahmann et al. (1966) [41] in connection to the disease resistance of higher plants, and a possible role of ethylene in resistance has been discussed. In another study, pine cells characterized by high ethylene production exhibited higher pox activity [42]. Moreover, ethylene induces the type III peroxidase gene (tcper-1) in cocoa [43].

Peroxidase activity produces oxidative power for cross-linking of proteins and phenylpropanoid radicals, resulting in reinforcement of cell walls against attempted fungal penetration [44]. Peroxidases are defense-related enzymes with a broad spectrum of activity. One of the induced resistance categories is systemic acquired resistance (SAR), which plays a central role in disease resistance. SAR develops either locally or systemically in response to a pathogen. It is associated with increased activity of lytic enzymes such as chitinases, b-1,3-glucanases, peroxidases, and other pathogenesis-related (PR) proteins, and also with accumulation of phytoalexins and lignin deposition [45]. They play key roles in plant–pathogen interactions, are believed to be one of the most important factors of the plant's biochemical defense against pathogenic microorganisms, and are actively involved in self-regulation of the plant's metabolism after infection [46]. PR-9 peroxidase is of the lignin-forming type and could be involved in the strengthening of cell walls [47]. In plants, peroxidase has also been linked with lignification of cell walls and is thought to be a factor in protecting stunted plants against other organisms through production of reactive quinones from phenolic compound catalysis [48].

4 Conclusions

The results of this study showed that the heights of maize plants treated with R_3, R_2, or R_4 did not differ significantly in comparison with R_0 treatment but did differ significantly in comparison with R_1 treatment. The numbers of leaves in maize plants

treated with R_4, R_2, or R_3 differed significantly in comparison with R_0 and R_1 treatment. The stem girths of maize plants treated with R_2, R_3, or R_4 did not differ significantly in comparison with R_0 treatment but did differ significantly in comparison with R_1 treatment. Peroxidase enzyme activity with R_0, R_2, R_3, or R_4 treatment was increased at 4 days and 8 days after inoculation; on the other hand, enzyme activity with R_1 treatment was increased only at 4 days after inoculation and was then decreased at 8 days after inoculation. The intensity of disease ratings with treatments R_0, R_1, R_2, R_3, and R_4 were about 2%, 28%, 10%, 9%, and 5%, respectively.

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