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Application of Mycorrhizaeinduced Resistance of Soybean Plants to Root Rotdisease (Sclerotium Rolfsii)

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Abstract. Stem rot caused by the fungus Sclerotium rolfsii is an important disease of soybean in Indonesia. This disease caused a significant yield loss averageof13-59%. This study aims to determine the ability of mycorrhizal to control stem rot disease in the two soybean cultivars. Fourdoses(0 g crop, 5 g crop, 10 g crop, and 15 g crop)of mixed mycorrhizal: Acaulosporasp, Glomus manihotis, and Gigaspora margarita (Mikofer) were applied to Anjasmoro and Kipas Merah soybean cultivars at planting time. Inoculation of S. rolfsiiwas carried out at 3 weeks after planting using a completely randomized design (CRD) with three replications. The variables observed: Incubation Period, Disease Incidence, Lesion Length, and Salicylic Acid Content. The results showed that the application of mixed mycorrhizae (micofer) was able to increase the resistance of soybean plants to stem rot disease. The percentage of affected plants decreased by 20.06% and 19.03% in the Anjasmoro and Kipas Merah cultivars respectively; the incubation period was 10.30 days and 10.44 days longer than the incubation period in non-mycorrhizal plants; the length of the lesions was shorter than the length of the lesion in the non-mycorrhizal plants. The salicylic acid content increased in both cultivars.

Keywords: Mycorrhizae, induced resistance, soybeans

1. Introduction

Stem rot (Sclerotium rolfsii) is the most common disease of soybean in Indonesia and causing large losses, especially during the rainy season or on poorly drained soil. In soybean, S. rolfsii infection causes a low percentage of seeds to germinate, thereby reducing plant populations. Moist land conditions are a conducive environment that can lead to severe S. rolfsii infections in soybean plants. The low resistance of the planted varietiesworsensthe disease incidence. Anjasmoro soybean variety was reported to be infected with S. rolfsii with a fairly high incidence of disease, reaching 23%, in contrast withWilis variety with the incidence of less than

10%(Fichtner, 2010; Rahayu, 2008). Damage to soybean plants at the age of 4 weeks after planting due to stem rot disease reached 33.03% in Aceh Besar district, Aceh Province(Marlina, Hakim, Alfizar, & Sufardi, 2008). This disease causes damage to the roots, stems, leaves, and fruit.

The use of mycorrhizal biological agents is an alternative way to develop a control measure of stem rot in soybeans. Mycorrhizae can form colonies on the roots of Gramineae, Leguminosae, and Solanaceae plants by utilizing photosynthate and/or root exudate as a source of nutrition. Besides being able to stimulate plant growth, mycorrhizae can also protect plants against pathogens by induced resistance mechanisms. It has been proven from several studies that mycorrhizae have a bioprotective effect against soilborne pathogens(St-Arnaud & Elsen, 2005; St-Arnaud & Vujanovic, 2007; Xavier & Bovetchko, 2004). According to Hoffland, Hakulinen, & Van Pelt, (1996) and Sutariati, Widodo, Sudarsono, & Ilyas (2005), induction of systemic resistance occurs due to mycorrhizal stimulation of plants to produce and accumulate compounds such as phytoalexin, chitinase protein, and salicylic acid which can inhibit the systemic penetration of several pathogens.

Induction of plant resistance is one of the biological control mechanisms that have the potential to be developed because of more practical use (applied to seeds/seedlings), efficiency (does not need to be repeated), economical and environmentally friendly(Habazar, 2004). This study aims to determine the ability of mixed mycorrhizal induction (micofer) against stem rot disease in soybean plants

2. Material And Methods

Inoculum propagation and plant material source

Sclerotium rolfsii fungus was isolated from soybean plants with symptoms of stem rot from farmers' land in BlangKrueng Village Aceh Province. The isolate was grown and purified on PDA media in the Plant Disease Laboratory of the Faculty of Agriculture, Unsyiah. The inoculum was subsequently preserved on PDA media in test tubes for 1 month until sclerotia wereformed.Mixed mycorrhizal inoculants: Acaulosporasp, Glomus manihotis, and Gigasporamargarita (micofer) were obtained from the Forest and Environmental Biotechnology Laboratory of IPB Bogor. The soybean seeds of Anjasmoro and Kipas Merah cultivars were obtained from BPTP Banda Aceh. Soybean plants were maintained in the greenhouse of the Faculty of Agriculture, Syiah Kuala University.

Experimental design

This study used a completely randomized design (CRD) with 3 replications. The treatmentswere 4 levels of mycorrhizal doses, namely: 0 grams per plant (control), 5 grams per plant, 10 grams per plant, and 15 grams per plant. Each treatment consisted of 3 plant pots so that the total number of plants was 36 experimental pots. Two plant pots were prepared for destructive plant samples for peroxidase analysis and salicylic acid content at 4 weeks after planting (MST).

Inoculation of the S. rolfsii Fungus

Pathogen inoculation was carried out on soybean plants 4 weeks after planting, by immersing the inoculum of S. rolfsii near the base of the stem as much as half of the test tubes containing mycelia and ten sclerotia grains. The plants then watered until theyreached the field capacity to retain moisture to promote the growth of sclerotia.

Application of mycorrhizal and NPK fertilizers

Mixed mycorrhizal inoculants (Micofer) were applied into the planting hole at the soybean planting date the dose according to the treatment. The seed was then thinly covered with soil. NPK fertilizer was given when the plants are 2 weeks after planting as much as 2 g per plant according to the recommended dose.

Variables observed

Disease Intensity

Observation of disease incidence was calculated when the plants had started to exhibit brown spots/spots at the base of the stem, calculated by the formula:

$P = (a / b) \times 100\%$

P = Percentage of plants attacked

a = Number of plants showing necrotic symptoms

b = Number of plants observed

Incubation Period

Observation of the incubation period was carried out every day after inoculation of S. rolfsii until the plant showed the first symptom, which was marked by brown spots on the base of the stem. Observations were started one day after S. rolfsii inoculation

Lesion length.

Lesion length was observed at 6 weeks after planting by measuring the length of the blackish-brown spot at the base of the stem.

Analysis of Salicylic Acid content

Salicylic acid content was analyzed using modified methods ofTenahaken & Rubel (1977)and Martinez dkk., (2000).

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3. Results and Discussion

Induction of soybean resistance to stem rot disease

The mixed mycorrhizae (micofer) tested in this study induced the resistance of soybean plants to stem rot disease caused by S. rolfsii. Even though the mycorrhizal plants were still attacked by stem rot disease, there was a decrease in the percentage of plants affected, an extension of the incubation period, and inhibition of the development of lesions length. This is related to the increase in salicylic acid content in soybean plants.

The results of laboratory analysis also showed that mycorrhizal fungi could increase the salicylic acid content in soybean plants. The salicylic acid content in Anjasmoroleavesreached 0.32 µg / g and 0.35 µg / g, in response of 10 g and 15 g mixed mycorrhizae (Mikofer) while in that of Kipas Merah reached 0.39 µg / g and 0.4 µg / g. The average salicylic acid content in the soybean plants of Anjasmoro and Kipas Merah cultivars which were induced by mycorrhizal resistance increased 2 - 3 times compared to control plants (Figure 1). This result was followingthe research results ofYalpani, Shulaev, & Roslanlian (1993),who stated that salicylic acid levels in tobacco leaves inoculated with CMV increased 70 times compared to before inoculated with CMV.

The application of mycorrhizae to plants will cause global changes in plants that will activate plant resistance genes and protect plants from pathogen attack (Bent, 1996; Ruiz-Lozano, Gianinazzi, & Gianinazzi-Pearson, 1999; Ruiz-Lozano, Roussel, Gianinazzi, &Gianinazzi-Pearson, 1999). This process involves various plant defense mechanisms related to changes in plant physiological and biochemical responses that involve the induction of hydrolytic enzymes such as peroxidase (Gianinazzi, Gianinazzi-Pearson, Tisserant, & Lemoine, 1992; Pozo, Azcon-Aguliar, Dumas-Gaudot, & Barea, 1999).

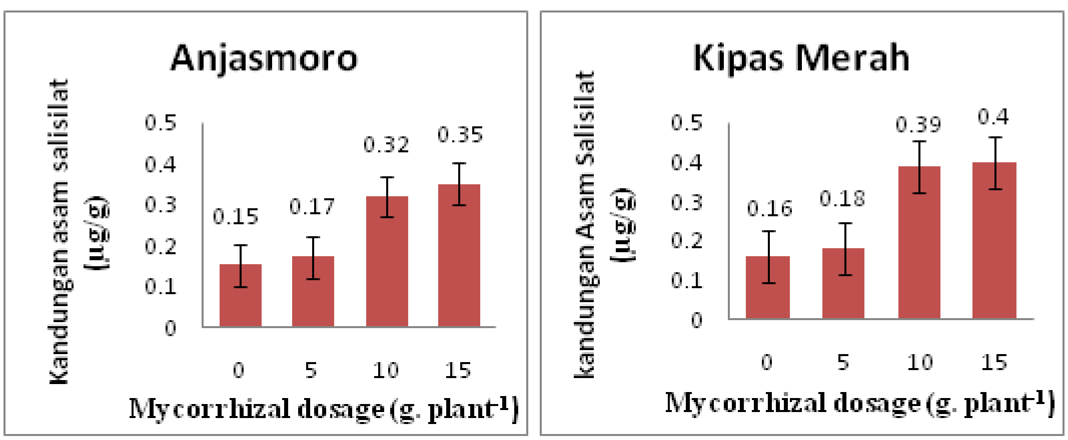

Figure 1.Mycorrhizal effect to Salicylic acid content in Anjasmoro and Kipas Merahcultivarswhichinduced resistance to stem rot disease.

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Disease Incidence, incubation period, and length of lesions

The effect of induced resistance can be seen from several disease parameters such as low disease intensity, longer incubation period, and delayed lesion length development.The application of mycorrhizal fungi on soybean plants reduced the percentage of soybean plants affected, prolonged the incubation period, and inhibited the development of lesions, both in Anjasmoro and Kipas Merah cultivars. The percentage of plants affectedbystem rot disease and the length of the lesions decreased as the dose of mycorrhizae was increased.The dose of 10 grams and 15 grams lower thedisease incidence of Anjasmoro cultivar to 10.3% and 10.35% or decreased by about 20.06%.Disease incidence ofKipas Merah was lower than that of Anjasmorowhen being applied by 10 gram and 15 gram which were 10,10% and 10,03%, decreasing as much as 19,03% (Figure 2.).

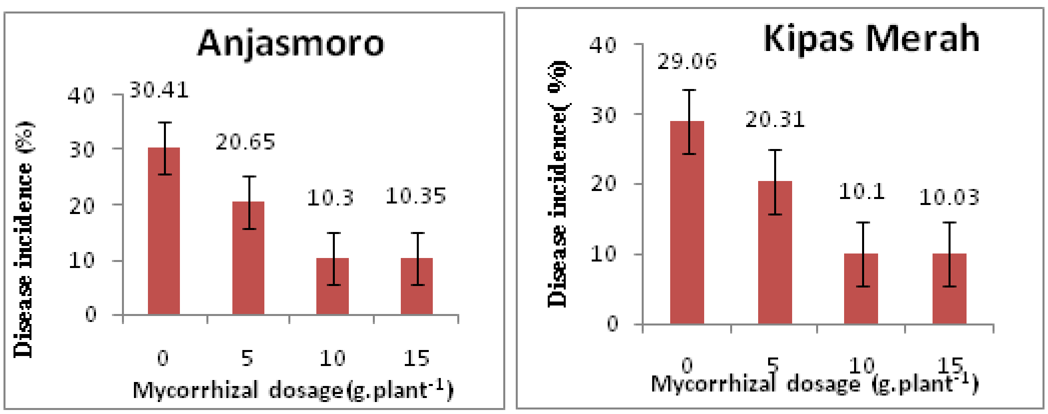

Figure 2.Disease incidence of stem rot disease atinduced soybean cultivars Anjasmoro and KipasMerahresistance to stem rot withmycorrhizae

The length of the lesions in the Anjasmoro cultivar applied with 10 g and 15 g mycorrhizae per plant were 1.50 cm and 1.45 cm or 0.56 cm shorter than the length of the lesions in soybean plants that were not given mycorrhizae (Figure 3). In the Kipas Merah cultivar, 10 g and 15 g of mycorrhizal application resulted in a lesion length of 1.22 cm and 1.05 cm or 0.75 cm shorter than the length of the lesion in control plants.

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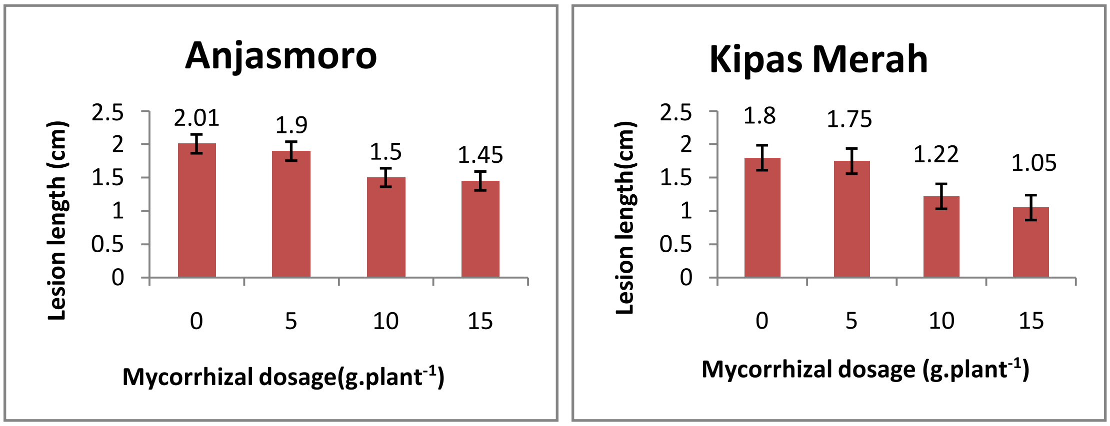

Figure 3. Length of the lesion in induced AnjasmoroandKipas Merah cultivarsresistance to stem rotwith mycorrhizae

The application of mycorrhizal fungi to soybean plants prolongs the incubation period of both Cultivar, Anjasmoro,andKipas Merah. The incubation period was delayed 11.12 days and 10.82 days after S. rolsii inoculation. (Figure 4.).

Salicylic acid was triggered to form in plants as a reaction to pathogenic infections. According to Hoffland et al., (1996)andSutariati et al., (2005), systemic resistance induction occur due to mycorrhizal stimulation of plants to produce and accumulate compounds such as phytoalexin, chitinase protein, and salicylic acid which can inhibit the systemic penetration of several pathogens.

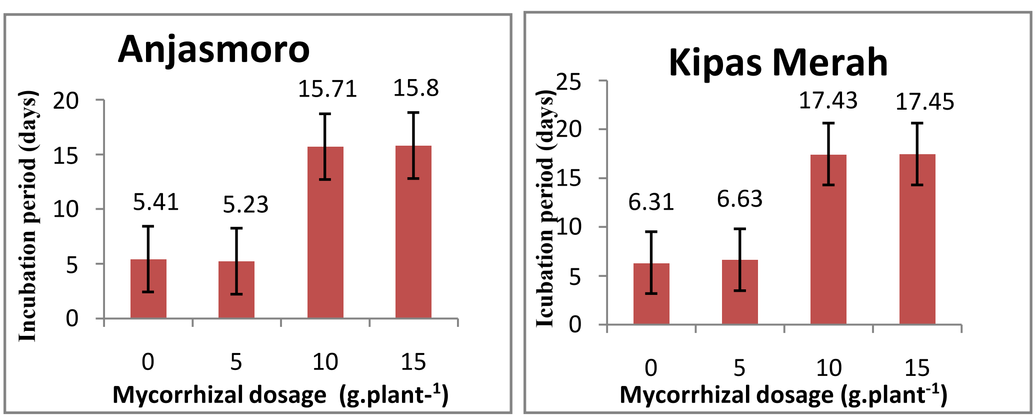

Figure 4.The incubation period of stem rot disease of soybeancultivarAnjasmoro and KipasMerah reduced their resistance withmycorrhizae.

Induced resistance in plants is related to the content of salicylic acid, which is a receptor that activates the formation of PR-protein(Pozo et al., 1999).The results of the correlation test showed that there was a moderate closeness (r = 0.47) between the variable length of the lesions

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and the salicylic acid content. In line with this statement,Murphy et al., (2001)stated that salicylic acid inhibits the systemic movement of pathogens from cell to cell throughout the plant.

Apart from salicylic acid, the peroxidase enzyme activity is reported to play a role in the mechanism of resistance to various pathogens in plants applied with mycorrhizal biological agents. Thus, in this study, it can be assumed that the application of mycorrhizae can increase the activity of peroxidase enzymes in Anjasmoro and Kipas Merah soybean plants. According toVan Loon, Pierpoint, Boller, & Conejero, (1994), peroxidase enzymes are a group of PR-proteins (pathogenesis-related protein) from the PR-9 group that accumulates in plants colonized by biological agents such as mycorrhizae. Peroxidase enzymes are compounds that catalyze the oxidation of hydrogen peroxide with lignin monomers such as r-cumaryl alcohol, coniferilalcohol, and alcohol synapses to become polymers in the form of lignin. The presence of lignin causes the plant cell wall to become thicker so that it is difficult for pathogens to penetrate (Hopkins, Webster, Chudek, & Halpin, 2001).

1. Conclusion

Mycorrhizal fungi can induce the resistance of Anjasmoro and Kipas Merah soybean plants to stem rot disease caused by *S. rolfsii*. Salicylic acid content increased 2-3 times in soybean plants which were induced by 10 g to 15 g mycorrhizal per plant. The increase in salicylic acid content had a positive correlation with the increase in the resistance of the two cultivars to stem rot disease.

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