Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Increasing the Activity of Defense Enzymes in Tomato Plants

Fadhila Rahmi Joni¹, Hasmiandy Hamid², Yulmira Yanti²

¹Post Graduated of Faculty of Agriculture, Andalas University, Padang, West Sumatera, Indonesia
²Department of Plant Protection, Faculty of Agriculture, Andalas university, Limau Manis, Pauh, Padang, West Sumatera, Indonesia

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Abstract— Growth-promoting rhizobacteria are non-pathogenic bacteria that can induce plant defense through induction of systemic resistance which can then activate defense enzymes such as Polyphenol Oxidase (PPO), Peroxidase (PO) and Penil Alanine Amoliase (PAL). This study aims to determine the activity of the PPO, PO and PAL defense enzymes with selected PGPR isolates. This research was carried out at the Microbiology and Greenhouse Laboratory of the Faculty of Agriculture, Andalas University, Padang, and then tested at the PAU IPB Laboratory, West Java Indonesia in March-July 2020. The results showed that tomato leaves that had been treated with PGPR showed that PGPR had the potential to increase the PO enzyme 0.072 µg · mL⁻¹, PPO 0.0009 µg · mL⁻¹ and PAL 14.15 µg · mL⁻¹. EAB 2.1 isolate is the best isolate can increase PPO, PO and PAL.

Keywords— PGPR, PPO, PO, PAL.

I. INTRODUCTION

PGPR is a non-pathogenic microbe that can increase plant fertility and induce plant resistance from biotic and abiotic stresses through the ISR mechanism. Plant Growth Promoting Rhizobacteria (PGPR) is a group of soil-specific microorganisms that efficiently colonize rhizosphere and rhizoplan and can substantially improve plant health (Hatami and Ghorbanpour, 2016). Specific mechanisms between PGPR and plant pests and pathogens are by producing antibiotics, competition of substrate and ecological niches, siderophores, chitinase enzymes, β-1,3-glucanase, cyanide, parasitism, and inducing systemic plant resistance (ISR) in the host. (Khalimi and Wirya. 2009).

ISR is an effective defense mechanism manifested as a result of physiological changes in plants, such as modifications to the cell wall structure and the synthesis of antimicrobial compounds such as proteins associated with pathogenesis (PR) and phytoalexins, which break the spread of pathogens (Filippi et al., 2011). Antioxidant enzymes such as peroxidase (PO), phenylalanine amonialyase (PAL) and polyphenol oxidase (PPO) may be enzymes elicited by ISR (Yasmin et al., 2016). Induced systemic resistance (ISR) involves the production of oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO), which catalyzes the formation of lignin, and other oxidative phenols that contribute to the formation of defenses (Meziane et al., 2005; Jetiyanon, 2007). PGPR can stimulate systemic responses in tomatoes by inducing the activity of defense enzymes such as phenylalanine ammonialyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and chitinase as well as the level of phenolic accumulation which further reduces infection by biotic pressure (Ahmed et al., 2011).

Several studies have shown the ability of PGPR to increase the activity of defense enzymes in plants, including cucumber roots treated with Pseudomonas corrugata 13 or Pseudomonas aureofaciens 63–28 can increase phenylalanine ammonia-lyase (PAL) activities, peroxidase (PPO) and polyphenol oxidase (PO) activity (Chen et al 2000). Turmeric plants introduced with Bacillus amyloliquefaciens BtNAU5 and Pseudomonas fluorescens strain Pf3TNAU triggered an increase defense enzymes peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), β-1,3-glucanase, chitinase, catalase and chemicals that trigger defense (total phenol) (Adhipati et al., 2014). In previous studies, there
were 3 root bacteria and 4 endophytic bacteria which were able to suppress whitefly populations and tomato wilt disease (Yanti 2018 and Hamid 2020). This study aims to determine the activity of the PPO, PO and PAL defense enzymes with selected PGPR isolates.

II. MATERIALS AND METHODS

The research was carried out in the Microbiology laboratory and greenhouses of the Faculty of Agriculture, Andalas University, Padang. Subsequently, a defense enzyme analysis was carried out at the PAU IPB Bogor Laboratory, West Java, Indonesia from March to July 2020.

Rejuvenation and Propagation of Rhizobacterial Isolates

Rhizobacterial isolates were obtained from the Yanti collection (2017). Rhizobacterial isolates were rejuvenated by means of one ose of bacteria transferred to NA medium in a Petri dish by the scratch method and incubated at room temperature for 24 hours. Furthermore, rhizobacterial multiplication was carried out consisting of 2 stages: (1) pre-culture, 1 rhizobacterial colony from pure culture was transferred into 10 ml NB medium in culture bottles and incubated on a rotary shaker at a speed of 150 rpm for 24 hours at room temperature. (2) Main-culture, 1 ml of suspension from preculture was transferred into 25 ml of sterile coconut water in a culture bottle and incubated in the same manner for 24 hours (Habazar et al., 2007). Rhizobacterial suspension from main-culture was determined by population density based on comparison with McFarland scale 8 (BaCl 0.8 g + H2SO4 1% 9.2 g) (bacterial population density estimated at 10^8 cells/ml) (Klement et al., 1990).

Preparation of Planting Media

Tomato growing media is a mixture of soil and sterile manure with a ratio of 2: 1. The mixture of soil and manure is sterilized for 1 hour at 100°C in a container measuring 45x40 cm2, then refrigerated for 24 hours. For the nursery, the soil is put into a pot-tray 22 grams / hole, while for planting chilies, the soil is put in a 4 kg / polybag

Introduction of rhizobacterial isolates

Rhizobacterial isolates were introduced twice, namely to seeds and seedlings.

Tomato seeds used are Warani Varieties. Tomato seeds are surface sterilized by soaking them in a 1% sodium hypochlorite solution for 3 minutes, after which they are drained and rinsed with distilled water 2 times and then dried. The seeds are soaked in rhizobacterial suspension for 15 minutes then planted in a pot-tray. Seedlings are maintained for 21 days.

Tomato seedlings that are 21 days old are transferred to polybags that have a mixture of sterilized soil and manure. Before planting, the roots are cleaned from the rest of the previous planting media and then immersed in rhizobacterial suspension for 15 minutes, then planting.

Measurement of Plant Defense Enzyme Activity

i. Peroxidase (PO)

The peroxidase enzyme activity test was performed by the Yanti method (2015). 1 g roots and stems macerated, then added 2.5 mL Potassium phosphate 0.5% buffer pH 7 and 0.1 g Polyvinyl pyrplidone (PVP). The suspension is homogenized and filtered using two layers of gauze, then centrifuged at a speed of 6,000 rpm (60 rpm = 1 Hz) for 15 minutes at 4°C. Supernatants are used to measure peroxidase activity.

Peroxidase activity measurements were carried out based on the Bateman method (1967). An enzyme extraction of 0.2 mL was added to a cup containing 5 mL pyrogallol (0.631 g pyrogallol in 0.005 M phosphate buffer pH 6, final volume 100 mL) and then shaken. The cup was placed on a spectrophotometer with 420 nm absorbance. 0.5 mL of 1% H2O2 is added to the cup, then shaken and immediately placed on a spectrophotometer. Changes in absorbance are observed every 5 seconds until there is no change. Peroxidase activity is expressed in μg/mL.

ii. Polyphenol oxidase (PPO)

1 g root and stem tissue samples were crushed and dissolved in 2 mL cold 50 mM phosphate buffer pH 6.5. The filtrate was then centrifuged at 16,000 g for 15 minutes at 4°C. The resulting filtrate is used as a source of enzymes. A 2.6 mL phosphate buffer solution pH 6.5 of 2.6 mL, 0.1 mL L-3,4-dihydroxyphenylalanine (L-DOPA) 5 mM, 0.1 mL ascorbic acid 2.1 mM, and 1 mL EDTA 0.065 mM is mixed until homogeneous. A crude enzyme of 0.1 mL was added to the solution and incubated for 10 minutes at room temperature. The solution was then measured for absorbance at a wavelength of 265 nm with a spectrophotometer (Karthikeyan et al., 2006).

iii. Phenylalanine Ammonia Lyase (PAL)

1g of leaf tissue samples were crushed and dissolved in 2 mL of cold 0.1 M sodium borate buffer pH 7. The filtrate was then centrifuged at 16,000 rpm for 15 minutes at 4 °C. The resulting filtrate is used as a source of enzymes. 2.0 mL of 3 mM L-phenylalanine solution was added with 0.9 mL of deionized water and mixed until homogeneous (Karthikeyan et al., 2006).
PAL activity was measured by a modified method of Sainders and McClure (1975). The reaction was carried out for 60 minutes at 37°C and an increase in absorbance at A290 nm were recorded at 15 min intervals. 200 mM Tris-HCl (pH 7.0) was used as a buffer solution and 20 mM L-phenylalanine as the enzyme test substrate. The rate curve of cinnamic acid formation was used as a measure of enzyme activity by using an absorbance increase of 0.01 at 290 nm as a cinnamic acid curve of 3.09 nmol. PAL activity was expressed in μmol of cinnamic acid min⁻1 g⁻1. Protein calibration was measured according to the Bradford (1976) standard method.

III. INDENTATIONS AND EQUATIONS

Tomatoes treated with PGPR showed higher defense enzyme activity when compared to controls. Peroxidase (PO) enzyme activity in tomato leaves introduced by PGPR showed a 3-4 times higher increase compared to the untreated control. Tomatoes treated with EAB 2.1 isolate showed the highest peroxidase levels with a value of 0.0698 μg · mL⁻¹ and the lowest was found in controls with a content of 0.0022 μg · mL⁻¹(Figure 1).

Apart from the PO enzyme, giving PGPR to tomatoes also increased the activity of the PPO enzyme. The PPO enzyme activity in tomatoes treated with PGPR also showed a 0.5-fold increase when compared to the control (Figure 2). The highest PPO was found in plants introduced by EAB 2.1 isolate with a PPO content of 0.095 μg · mL⁻¹, while the lowest was found in the control with a value of 0.045 ppm. All isolates used in this study showed enzyme activity above the control plants.

Furthermore, PGPR treatment on tomato plants can increase the activity of the enzyme Penyilanalin Ammonia lyase. The activity of the PAL enzyme given PGPR can increase PAL by 0.17μg · μg · mL⁻¹. The highest PAL activity was found in EAB 2.1 isolates with a value of 15.98 μg · mL⁻¹, while the lowest was found in controls with a content of 13.98 μg · mL⁻¹ (Figure 3). This is because PGPR can increase the level of peroxidase activity in plants.

Giving PGPR to tomato plants shows the induction mechanism of systemic resistance in tomatoes which is then expressed through the activation of defense enzymes such as PO, PPO and PAL. Ahmed et al., (2011) stated that PGPR is stimulating Systemic response in tomatoes by inducing high levels of enzymes activity of phenyalanine ammonialyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and chitinase as well as accumulation of high levels of phenolics. In the study, it was found that there was an increase in defense enzymes induced by PGPR in tomato plants with a 3-4-fold increase in PO, 0.5-fold PPO and 2-fold PAL. This was also found by Ahmed et al., (2011) who reported that the administration of PGPR P. puptida and P. flureccens to tomato plants could increase PAL activity 3 times, PO 2 times and PPO 2 times.

Polyphenol oxidase is a plant defense enzyme from biotic and abiotic stress. Polyphenol oxidase is an enzyme in plants that regulate feeding, growth, development of insect pests, and play a major role in plant defense against biotic and abiotic stresses (Sharma et al., 2009). PO is a group containing enzyme copper that catalyzes the oxidation of hydroxy phenols to derivative quinones, which have antimicrobial activity (Chunhua et al., 2001). In the research that has been done, the PO enzyme can increase 3-4 times and all isolates in the treatment showed enzyme activity above the control. This shows that all PGPR isolates used in this study have the potential to increase PO levels. Research conducted by Sharavankumar et al., (2006) states that giving PGPR to tea plants can increase the activity of the PPO enzyme. An increase in PO levels also occurred in rice plants treated with Bacillus spp (Rais et al., 2017).

Furthermore, there is the peroxidase enzyme which is one of the important enzymes in defense that plays a role in strengthening plant cell walls. Peroxidases produce reactions that regulate defense-related signal transduction pathways, initiate hypersensitivity reactions, and strengthen cell walls through increased lignification (Ralph et al., 2004). In the research, the peroxidase enzyme activity in untreated tomato plants was only around 0.0002 μg · mL⁻¹ whereas those treated with PGPR could reach 0.0009 μg · mL⁻¹. This indicated that there was an increase in PO activity in plants treated with PGPR and all isolates showed more increases than the control. This is also obtained by Sarvanan et al., (2004) that the increase in PO activity in banana tubers introduced with Psuedomonas fluorescence and suppressed fusarium wilt disease. Rice plants induced by Bacillus spp can increase polyphenol oxidase enzymes as much as 3.0–3.8 fold and can suppress development Pyricularia oryzae (Rais et al., 2017).

PAL is one of the important enzymes that play a role in other phenolic synthesis related to plant defense (Daayf et al., 1997). PAL serves as a precursor file for the biosynthesis of lignin and other phenolic compounds that accumulate in response to infection (Klessing and Melany 1994). In studies that have been carried out PAL activity on treated tomatoes can increase up to 0.17 μg · mL⁻¹ when compared to controls. Although there is only an increase 0.17 μg · mL⁻¹ but all isolates used in this study
had the potential to increase PAL activity in tomatoes. Yanti (2015) also reported that onions introduced with 8 rhizobacteria isolates showed an increase in peroxidase enzyme activity in the roots and leeks, PK2Rp3 was the best isolate with an increase in enzyme activity reaching (0.058 µg · mL⁻¹) in roots and leaves.

IV. FIGURES AND TABLES

Enzim Peroksidase

Fig. 1: Production of Peroksidase in tomato plants introduced by PGPR

Enzim Polifenol Oksidase (PPO)

Fig. 2: Production Polifenol Oksidase enzymes in tomato plants introduced by PGPR
Fig. 3: Production of Phenilalanin Ammonia Lyase enzymes in tomato plants introduced by PGPR

V. CONCLUSION
PGPR can induce plant resistance through ISR which further increases the activity of the PO, PPO and PAL enzymes. Seven isolates used in this study were able to increase the activity of the PO, PPO, and PAL enzymes, with Isolate EAB 2.1 being the best isolate in increasing enzyme activity.

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