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Growth Performance and Metabolic Changes in Susceptible Mung Bean [*Vigna radiata* (L.) Wilczek] during Interaction with *Rhizoctonia solani* and *Trichoderma virens*

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ABSTRACT

Background: Mung bean is susceptible to *Rhizoctonia solani* infection. Applications of beneficial microorganisms such as *Trichoderma* are promising for controlling pathogens and promoting plant growth.

Methods: This study investigated growth performance and metabolic changes in mung bean seedlings during interaction with *R. solani* and *Trichoderma virens* using Gas Chromatography-Mass Spectrometry (GC-MS).

Result: Mung bean infected by *R. solani* caused root rot and wilting. *T. virens* treatment reduced the disease severity in infected seedlings and promoted mung bean growth. Seventy-eight metabolites were identified in root extracts and dominated by sugars and fatty acids. The sugars, fatty acids and organic acids were significant metabolite groups that changed in response to pathogen infection and/or *T. virens* treatment. Five metabolic pathways particularly pyruvate metabolism, glyoxylate and dicarboxylate metabolism, sulfur metabolism, citrate cycle (TCA cycle) and phenylalanine, tyrosine and tryptophan biosynthesis altered significantly based on a metabolic pathway analysis. Acetic acid and aconitine had important roles in mung bean response to *R. solani* infection and/or *T. virens* treatment.

Key words: GC-MS, Metabolic profile, Mung bean, *Rhizoctonia solani*, *Trichoderma virens*.

INTRODUCTION

Mung bean is one of the substantial legume crops which is rich in protein. Unfortunately, this commodity is prone to soil borne diseases. *R. solani* is devastating pathogens on mung bean crops in Indonesia (Rahayu, 2016). Currently, the use of beneficial microbes such as *Trichoderma* is widely used due to their ability to control soil borne pathogens (Xue *et al*., 2018) and induce plant systemic resistance as well as promote plant growth (Harman, 2011; Surekha *et al*., 2013; Mayo-Prieto *et al*., 2019).

Plant-pathogen-*Trichoderma* interactions are complex and specific systems. Recently, a metabolomics approach has been intensively studied to clarify the mechanisms underlying interactions between beneficial microbes and plant pathogens. The metabolomics is able to describe plant metabolic changes as a response to pathogen infections and biological control agent applications (Allwood *et al*., 2010; Hu *et al*., 2017). Rojas *et al*. (2014) reported that primary and secondary metabolites involve in crop resistance.

An interaction of *Trichoderma* sp. with *R. solani* in *Phaseolus vulgaris* has been thoroughly investigated from genetic to protein levels (Mayo-Prieto *et al*., 2019). However, information of metabolic studies on legumes is still limited. Pathogen infection and/or *Trichoderma* sp. trigger the changes of metabolic expression in bean plants (Mayo-Prieto *et al*., 2019). Evidence of changes in mung bean metabolites caused by *R. solani* infection and bio-control agent interaction remains unexplored. Therefore, this current study aimed to observe the growth performance and metabolic changes in mung bean seedlings during *R. solani* infection and/or *T. virens* treatment.

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MATERIALS AND METHODS

The experiment was conducted at a greenhouse of Indonesian Legume and Tuber crops Research Institute and Central Laboratory of Indonesian Agency for Agricultural Research and Development, Malang, East Java, Indonesia in 2019. Mung bean was grown on sterile soil in plastic pots. Seeds were surface sterilized using 0.5% NaOCl and dipped into conidia suspension of *T. virens* (1×10^6 CFU mL^{-1}) for 30 minutes. Mung bean seeds dipped into sterile water were used as a control. Four different treatments, *i.e.* (1) plants infected by *R. solani* (Rs), (2) plants infected by *R. solani* and treated with *T. virens* (RsTv), (3) plants treated with *T. virens* (Tv) and (4) control plants without *R. solani* and *T. virens* (C) were tested. The treatments were arranged in a

randomized completely block design with ten replications. The seedlings were harvested at 21 days after planting (dap). The percentage of post-emergence damping-off was observed at 21 dap. Disease severity of *R. solani* infection was assessed using a scale in Porto *et al*. (2020) study.

Metabolite analysis using gas chromatography-mass spectrometry (GC-MS)

Samples for metabolic analysis were prepared in accordance with Lisec *et al*. (2006) with modifications. Centrifugation at 11,000 g for 10 minutes was conducted to separate the extracts. The supernatant was evaporated using nitrogen flow. Two steps of derivatization using 40 µL methoxyamine hydrochloride in pyridine and 40 µL N,O-bis(trimethylsilyl) trifluoroacetamide in 5% trimethylchlorosilane were performed. The derivatized samples were analyzed using gas chromatography-mass spectrometry (Trace 1310-ISQ LT, Thermo Scientific).

Data analysis

Data obtained from GC-MS were evaluated using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/home.xhtml). To determine the function of metabolites, openly available metabolite libraries including plant metabolic network (PNM) database (https://plantcyc.org), PubChem (https://pubchem.ncbi.nlm.nih.gov/) and KEEG pathway database (https://www.genome.jp/kegg/pathway. html) were used.

RESULTS AND DISCUSSION

Growth performance of mung bean seedlings

Plant-pathogen-*Trichoderma* interaction affected mung bean seedling growth and development. *R. solani* infection caused rotten seeds and roots as well as damping-off. Disease incidence was the highest in *R. solani* treatment in contrast to *T. virens* treatment (Table 1). Low disease severity observed in *R. solani* infection and the presence of *T. virens* (RsTv) was due to direct antagonistic activity and plant growth promotion of *T. virens*. Similar results were reported by Godara and Singh (2021) where *Trichoderma* reduced the occurrence of moth bean root rot and significantly increased grain yield.

T. virens treatment increased plant growth particularly root length and fresh biomass (Table 1). The application of *T. virens* increased plant capacity to absorb nutrient through root elongation and biomass allocation at the early phase of mung bean growth. During plant-microorganism interactions, *Trichoderma* activate chemical signaling and metabolite regulations that lead to plant systemic defense response as well as growth regulation (Rojas *et al*., 2014).

Composition of mung bean metabolites

Seventy-eight metabolites were identified in the mung bean root extracts. Based on their functional groups, those metabolites consisted of sugars (38%), fatty acids (15%), sugar alcohols and alcohols (10%), organic acids (10%), amino acids and their derivatives (4%) and other compounds including phenols, sterols, aldehydes and ketones (23%) (Fig 1).

Sugar compositions in mung bean infected by *R. solani* (Rs) were comparable to those in infected plants and *T. virens* treatment (RsTv) and control plants (C). Sugar production is carbon source and energy in plant primary metabolism besides as signaling in hormonal coordination pathways (Rojas *et al*., 2014). Sugar is also one of the key compounds in abiotic stress adaptation mechanisms (Yadav and Hemantaranjan, 2017). Fatty acids and organic acids in infected and *T. virens* treatment (RsTv) were higher than those in control plants (C). Aldehydes, ketones, amino acids and other miscellaneous compounds were higher in mung bean treated with *T. virens* (Tv). Fatty acids and lipids are essential for cells, as energy for metabolic process and signals for both intracellular and extracellular to trigger immunity (Walley *et al*., 2013, Lim *et al*., 2017). Amino acids are crucial for growth, development, stress responses and the immune system of plants (Kadotani *et al*., 2016).

Metabolic profiles and changes of metabolites during plant-pathogen-*Trichoderma* interaction

A heatmap cluster analysis representing metabolic profiles of mung bean roots grouped the treatments into two main clusters (Fig 2). Pathogen infection and *Trichoderma* treatment (RsTv) was grouped into different cluster with other treatments (Rs, Tv and C). The presence of the pathogen in combination with the antagonistic fungi (three-way interaction) induced higher relative plant metabolite expression than that in two-way interactions (plant-pathogen or plant-*Trichoderma*).

R. solani infection increased the accumulation of several sugars such as D-ribose, tagatose, allose, lyxose and xylose. In contrast, pathogen infection reduced several compounds such as myo-inositol, acetic acid, D-manitol, D-ribose and D-galactopyranoside. Those accumulated metabolites are pathogenesis-related (PR) metabolites which are produced

Table 1: Disease incidence and growth parameters of mung bean seedlings treated with *R. solani* and *T. virens*.

Treatment	Disease incidence (%)	Disease severity	Growth parameter		
			Root length (cm)	Plant height (cm)	Fresh biomass plant^{-1} (g)
Rs	48.72±3.8	2.60±0.16	14.50±0.5	11.94±0.8	1.53±0.14
RsTv	11.10±1.3	0.23±0.02	22.79±0.8	13.30±0.3	1.65±0.08
Tv	2.89±0.7	0.44±0.04	26.91±1.5	12.52±0.3	2.04±0.22
C	0	0	21.50±1.8	13.35±0.2	1.69±0.12

Note: Rs= Plants infected by *R. solani*, RsTv= Plants infected by *R.solani* and treated with *T. virens*, Tv= Plants treated with *T. virens* and C= Control without *R. solani* and *T. virens*.

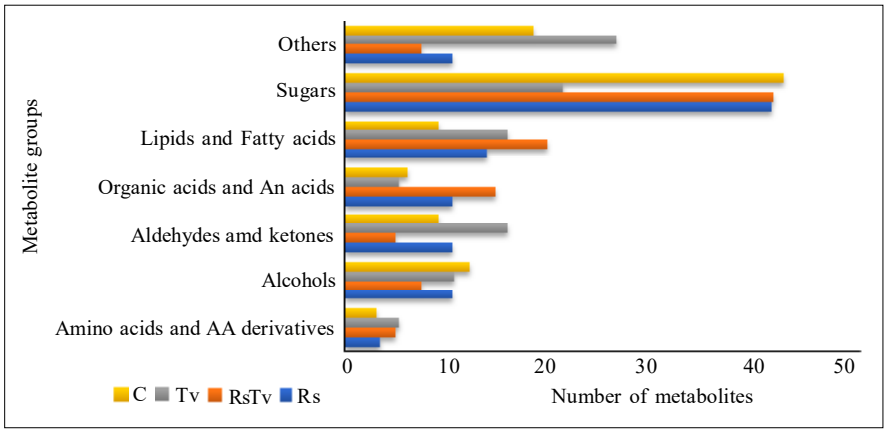

Fig 1: Metabolite compositions in mung bean roots in different treatments. Rs= Plants infected by *R. solani*, RsTv= Plants infected by *R. solani* and treated with *T. virens*, Tv= Plants treated with *T. virens* and C= Control without *R. solani* and *T. virens*.

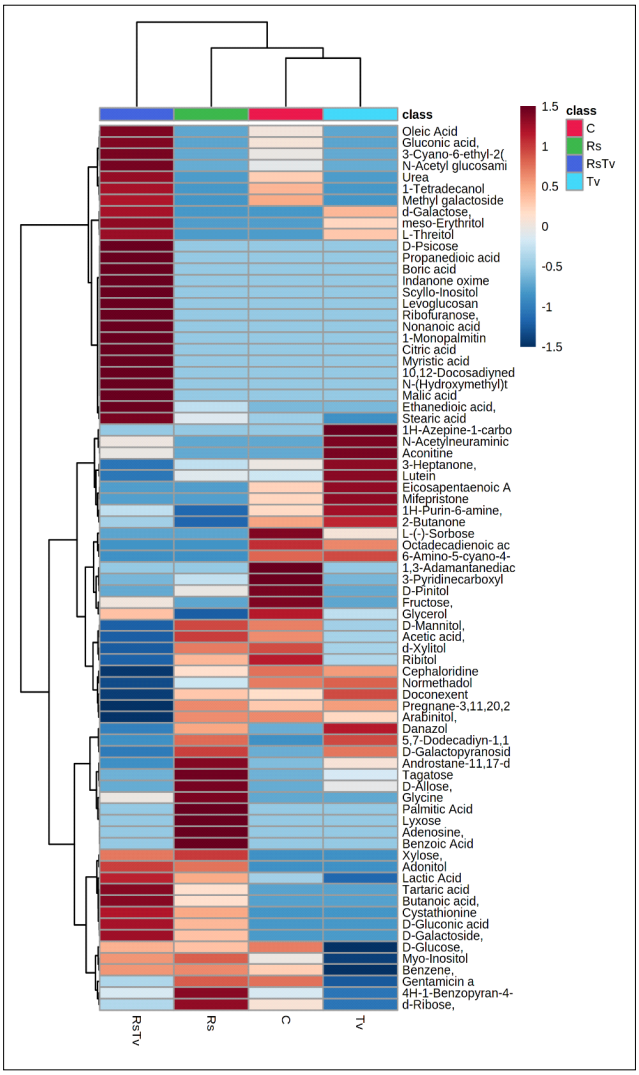

Fig 2: Heatmap of metabolite contents in mung bean seedlings infected by *R. solani* and *T. virens* treatment. Rs= Plants infected by *R. solani*, RsTv= Plants infected by *R. solani* and treated with *T.virens*, Tv= Plants treated with *T. virens* and C= Control plants.

after pathogen infection and antimicrobial activities (Chahed *et al*., 2021) and some responsible for resistance against pests (Reddy *et al*., 2021).

Higher intensities of fatty acids such as ethanedioic and stearic acids were observed in all treated plants. Ethanedioic acid (syn. oxalic acid) is produced by both plants and fungi (Prasad and Shivay, 2017). Fatty acids have important roles in both biochemical and physical mechanisms of plant resistance through the production of guard cells (Nakata, 2015).

Changes of amino acids levels were also observed during the plant-pathogen interaction that affected mung bean growth and resistance to pathogens. Purine was detected in both control and treated plants; however, cystathionine and glycine were only presence in the treated plants. Purine and cystathionine are members of cytokinin which promote plant growth and as substrate for microbial growth and development (Schläpfer *et al*., 2017).

Experimental treatments and metabolic production relationship

A principal component analysis (PCA) and partial least-square discriminant analysis (PLS-DA) were conducted in determining the relationships between experimental treatments and accumulation of metabolites from un-supervised analysis of GC-MS data. Two principal components (PC1 and PC2) showing three-way interaction of mung bean, *R. solani* and *T. virens* (RsTv) were the most dominant variable affecting the variability of mung bean metabolic expressions (Figure 3a). Based on the PC value, twelve metabolites were responsible for the interaction of mung bean- *R. solani* - *T. virens,* namely acetic acid, docosadiynedioic acid, heptanone, pyridine carboxylic acid, benzopyran-4-one, 6-Amino-5-cyano-4-(5-cyano-2,4-dimethyl-1H-pyrrol-3-yl), adenosine, benzoic acid, D-allose, D-mannitol, D-ribose and lyxose.

Variable importance in projection (VIP) values from PLS-DA analysis showed that 13 metabolites had scores higher than 1.5 (Fig 3b). Metabolites of 3-heptanone, aconitine, N-acetylneuraminic acid, ethanedioic acid, lutein and H-Azepine-1-carboxylic acid were detected higher in *T. virens*

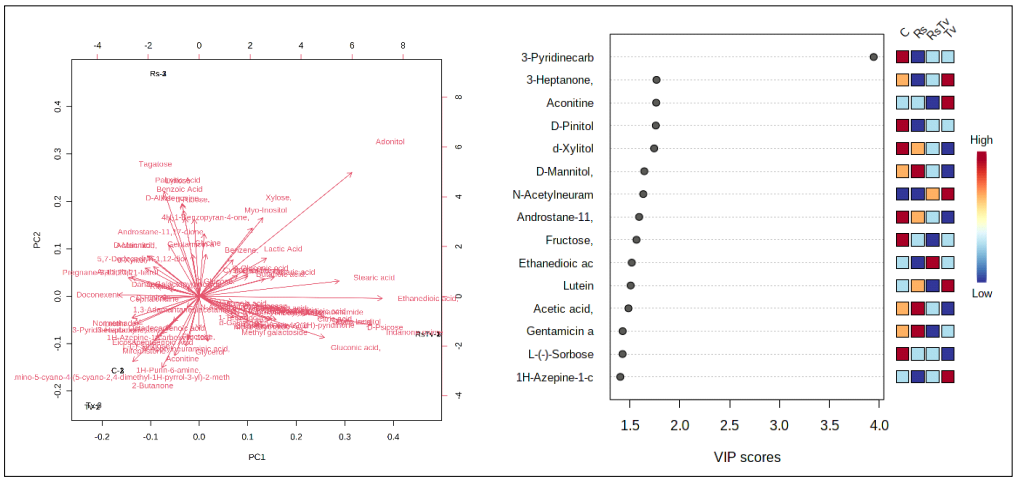

Fig 3: (a) Biplot analysis of PCA and (b) VIP-values of significant metabolites in mung bean roots.

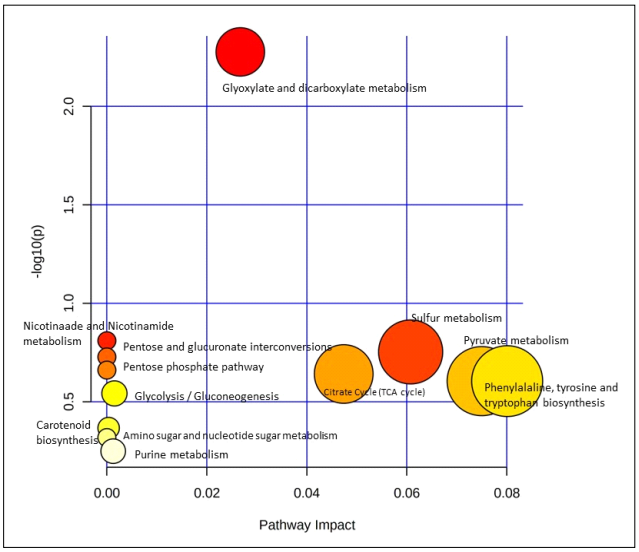

Fig 4: Metabolic pathway analysis plots depict several metabolic alterations in mung bean. The different color of each circle is based on p-values; darker color and bigger size indicate significant higher changes of metabolites in the corresponding pathway.

treatment. Meanwhile, D-mannitol, acetic acid and gentamicin were higher in infected mung bean seedlings.

Metabolic pathway analysis was carried out using 23 significant metabolites from the PCA and PLS-DA values. Pathogen infection and/or *Trichoderma* treatment (Rs, Tv and RsTv) affected 12 metabolite pathways on mung bean networks (Fig 4). Five metabolic pathways showed significant high impact indicating by darker color and bigger circle in the pathway analysis plot namely, glyoxylate and dicarboxylate metabolism, sulfur metabolism, citrate cycle, pyruvate metabolism and phenylalanine tyrosine and tryptophan biosynthesis.

Further analysis of each metabolite of the twelve significant pathways showed that several overlapping metabolites performed as a link metabolite that was capable of connecting various pathways. In this study, acetic acid and aconitine which involved in more than one significant pathway were affected by all treatments. Acetic acid involved in three metabolic pathways, namely glyoxylate and dicarboxylate metabolism, glycolysis/gluconeogenesis biosynthesis and sulfur metabolism. In addition, aconitine involved in glyoxylate and dicarboxylate metabolisms as well as TCA cycle. Glyoxylate and dicarboxylate metabolism and glycolysis/gluconeogenesis involve in carbohydrate metabolism (Zhang *et al*., 2019). Carbohydrates increase resistance to biotic and abiotic stressors indirectly through supplying energy for growth (Saddhe *et al*., 2021). In this study, *T. virens* (Tv) triggered the elevation of aconitine compared to that in control, whereas pathogen infection (Rs) increased the level of acetic acid. Both pathogen and *Trichoderma* affected the accumulation of important metabolites in carbohydrate biosynthesis. Our study also suggested that sulfur and pyruvate metabolisms performed the highest pathway impact, meanwhile, purine metabolism showed the least affected (Fig 4). Several studies reported that sulfur metabolism has a crucial role in determining crop growth and fitness towards microbial and viral infections as well as plant resistance to environmental stress (Aziz *et al*., 2016). This finding showed that acetic acid and aconitine had important roles in mung bean metabolic networks.

CONCLUSION

R. solani infection and/or *T. virens* treatment affected mung bean seedling growth. These interactions changed the metabolic profiles in mung bean seedlings especially three metabolic groups of sugars, fatty acids and organic acids. In this study, pathogen infection and/or *T. virens* treatment influenced 12 metabolic pathways. Five pathways showed significant high impact, *i.e*., glyoxylate and dicarboxylate metabolism, sulfur metabolism, citrate cycle (TCA cycle), pyruvate metabolism and phenylalanine, tyrosine and tryptophan biosynthesis. Altered metabolite pathways under pathogen infection and/or *Trichoderma* treatment associated with pathogenesis-related metabolites and signaling for plant defense reaction. This present study suggested that two

metabolites of acetic acid and aconitine had important roles in mung bean metabolic networks under plant-microbe interactions.

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Conflict of interest

The authors have no conflicts of interest to declare regarding the publication of this present paper.

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