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IAA AND GA_3 ASSAYS OF RHIZOSPHERE FUNGI FROM JABON MERAH (*NEOLAMARCKIA MACROPHYLLA*) STAND OF SIDRAP PROVENANCE, SOUTH SULAWESI

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ABSTRACT : Fungi have been shown to synthesize various plant hormones, including gibberellin, abscisic acid (ABA) and cytokinin. This study aimed to determine the ability of rhizosphere fungi from the jabon Merah stand to produce IAA and GA_3. Samples were collected from the jabon Merah stand of Sidrap provenance, South Sulawesi. IAA and GA3 assayed of the fungi were carried out at Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin. Eight fungi isolates were used in the experiment. IAA assay showed that the highest IAA concentration was from JCS 6 isolate, 17.95 ppm. Similarly, JCS 6 isolate also had the highest GA_3 concentration (4.20 ppm) by GA_3 assay. This study will be followed by macroscopic and molecular identification of the JCS 6 isolate. The detection is needed in order to develop the mass production of fungi as biological agents.

Key words : IAA assays, GA_3 assays, fungi, *Neolamarckia macrophylla.*

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INTRODUCTION

The rhizosphere is a layer of soil covering the surface of plant roots that is still influenced by surface root activities and acts as an external defense for plants against root pathogens. This soil layer shows the part of the soil affected by plant roots, which is characterized by more microbiological activities compared to in the soil located deeper from the roots. The rates of metabolic activities of rhizosphere microorganisms are different from that of non-rhizosphere soil (Rao, 2010).

The presence of plant roots mainly determines the important role of the rhizosphere. The more and denser plant roots in the soil, the richer the content of organic matter in the rhizosphere, and the denser the population of soil microbes. One of the microbes that inhabit the soil is a fungus. Soil fungi can survive in an unavailable host state as a saprophyte on decayed or dormant organic matter in the form of spores or sclerotium (Tambingsila, 2016).

Gibberellin (GA) and indole-acetic acid (IAA) are important secondary metabolites that are commercially produced from fungi for the agricultural and horticultural industries (Hasan, 2002). IAA and GA_3 have in common, namely increasing cell elongation, flowering, and parthenocarpy. The difference between both hormones is that the auxin is polar transport, while gibberellin transport is nonpolar. Based on these two statements, it can be assumed that auxin and gibberellins can work solely or together, depending on the type of plant and environmental conditions of plant growth. Gibberellins play a role as hormones in plants, affecting growth and differentiation in highly regulated organs (Hedden, 2020).

Based on the above considerations, the identification of rhizosphere fungi isolates on jabon Merah (*Neolamarckia macrophylla*) is a wood-producing tree as a community and industrial forest plant because of its characteristics (fast-growing, deciduous, and self-pruning) and high adaptability to various environmental conditions has good prospects (Danu and Subiakto, 2015; Mompewa *et al*, 2019). Jabon Merah has the potential to be developed and conserved (Arif *et al*, 2019; Batti *et al*, 2020;

Larekeng *et al*, 2019). Thus, the information about the potential rhizosphere fungi producing IAA and GA_3 hormones is needed later as plant growth formulations to increase the optimal growth of superior plants and forest plant seedlings.

MATERIALS AND METHODS

The soil samples collection was conducted at Jabon Merah stand in Pitu Riase, Sidrap district, South Sulawesi, Indonesia. In vitro isolation and identification were at Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin, Makassar, Indonesia. The research materials were rhizosphere soils, PDA (Potatoes Dextrose Agar) medium, PDB (Potatoes Dextrose Broth) medium, chloramphenicol, sterile aquadest, salkowski solution, 70% alcohol, agar, heat-resistant plastic, aluminum foil, and so on.

Collection of soil samples and Isolation of soil fungi

Soil samples collection were performed by collecting soil at three points of each location at a depth of 5-20 cm and as many as 10 grams and then put into a sample container (given the sample code identity). The samples were then stored at a -300C up to -400C in the freezer at the laboratory. The microbial isolations were carried out using the dilution method by making a series of dilutions (10^{-1} and 10^{-2}). The medium used was a modified PDA medium with antibiotics for growing and isolating the fungi. The incubation process was at room temperature of 27°C for ± five days and the growth was observed. The selection of purified microbial colonies was based on the differences in the colony morphology in terms of color, elevation, surface texture so that pure isolates were obtained. Purification of fungal isolates was performed by moving the fungi using the point method on the media (Elias *et al*, 2016).

Indole Acetic Acid assay

Screening of IAA-producing fungi was tested qualitatively by colorimetric using Salkowski reagent (Iradhatullah *et al*, 2015). The positive isolates for the qualitative test were then continued with quantitative analysis using the spectrophotometric method (Gravel *et al*, 2007). The isolates were grown on PDA media and added with tryptophan and then incubated. After incubation, the absorbance value of the supernatant was measured using a UV-Vis Spectrophotometer with a wavelength of 520 nm. This wavelength was chosen based on the color produced by the interaction between Salkowski's reagent and IAA (Glick, 1995), which produces a pink color. The absorbance results were entered into the IAA standard curve equation. The IAA concentrations of the samples were calculated based on a standard curve with pure IAA standards (Gravel *et al*, 2007).

Gibereline Acid (GA_3) assay

GA_3 hormone-producing fungi were measured using the (Borrow *et al*, 1955) method. Fungal isolates were grown on PDB media and incubated for seven days, and then shake. The supernatant was taken as much as 5 ml, added 5 ml of 30% chloric acid, and incubated at room temperature for 74 minutes. The absorbance was measured using a spectrophotometer at a wavelength of 254 nm and the blank using 5% chloride acid. The GA_3 concentrations of the samples were calculated based on the standard curve with the GA3 standard.

RESULTS AND DISCUSSION

Macroscopic identification showed that eighteen isolates of the fungi were found in the rhizosphere of jabon Merah. The morphological characterization of eighteen fungal isolates identified the variations in color, texture, and surface elevation of the fungi. The colors of the colony were dominated by green and white, while the texture majority were velvety and cotton-like. The macroscopic characteristics of each fungal isolate are described in Table 1.

The phytohormones production assays and physiological characterization on eighteen isolates of fungi showed the capability to produce phytohormones IAA and GA_3. The concentrations of IAA and GA_3 produced by isolates are presented in Table 2. The eighteen rhizosphere fungi isolates had the capability to produce IAA with concentrations of 1.05 ppm to 17.95 and GA3 production with concentrations of 2.50 ppm to 4.20 ppm.

The capability of the microbes to produce IAA and GA_3 hormones is influenced by species. The IAA production capability assay on eighteen rhizosphere fungi isolates showed the variation in the color. The color change indicates the capability to produce IAA, the darker the color produced after adding the Salkowski reagent solution, the higher the IAA concentration produced by the fungi.

The highest IAA production was produced by JCS6 isolate with 17.95 ppm IAA, followed by JCS4 (2.52 ppm), and the third-highest was JCS 16 (1.88 ppm). All the evaluated fungi were also able to produce Gibberellin (GA_3) hormone. Gibberellins are the growth hormones that play vital roles in stimulating cell division, stem elongation, flowering, and fruit growth. JCS6 isolates produced the highest concentration of GA_3 hormone compared to other fungi, 4.20 ppm, followed by JCS18 isolate (4.16 ppm) and JCS 15 (4.05 ppm). Based on the IAA and GA_3 produced by Rhizosphere fungi isolates,

Table 1 : The macroscopic characteristics of the fungal isolate from Jabon Merah rhizosphere.

Sample code	Macroscopic morphology			
	Top	**Base**	**Texture**	**Surface**
JCS 1	White margin, green center	Yellow	Velvety	Flat
JCS 2	White margin, green center	White	Velvety	Flat
JCS 3	White margin, green center	White, Yellowish	Velvety	Flat
JCS 4	Greenist white	Greenist white	Cotton-like	Convex
JCS 5	White	Brownist white	Cotton-like	Convex
JCS 6	Greenist white	White, green, yellowish	Cotton-like	Convex
JCS 7	Green	Yellowish white	Velvety	Flat
JCS 8	Greyish black	White	Velvety	Flat
JCS 9	White green	Greenist	Cotton-like	Convex
JCS 10	Greyish black	White	Velvety	Flat
JCS 11	White margin, blueish green	Yellowish white	Velvety	Flat
JCS 12	White	White	Cotton-like	Flat
JCS 13	Green	White green	Velvety	Flat
JCS 14	White margin, yellow center	Brown yellow	Velvety cotton-like	Flat
JCS 15	Greenist white	Greenist white	Cotton-like	Convex
JCS 16	Blues white	Brownist white	Fine cotton-like	Flat
JCS 17	Brownist white	Brownist white	Cotton-like	Dome-shape
JCS 18	White margin, white green center	Yellowish white	Velvety cotton-like	Convex

Table 2 : Physiological characterization of fungi isolate.

Sample code	GA_3 (ppm)	IAA (ppm)
JCS 1	4.02	1.14
JCS 2	3.87	1.33
JCS 3	3.84	1.27
JCS 4	3.38	2.52
JCS 5	4.03	1.11
JCS 6	4.20	17.95
JCS 7	4.04	1.05
JCS 8	3.16	1.38
JCS 9	3.35	1.70
JCS 10	2.89	1.56
JCS 11	2.48	1.72
JCS 12	2.50	1.23
JCS 13	3.99	1.23
JCS 14	3.70	1.05
JCS 15	4.05	1.23
JCS 16	3.88	1.88
JCS 17	2.88	1.20
JCS 18	4.16	1.16

the most potential rhizosphere fungus in producing phytohormones were the JCS6 isolate.

The IAA produced by Trichoderma isolates was 9,656 ppm with an incubation period of 3 days, but after seven days of incubation, the IAA concentration decreased to 4,049 ppm. The 17,95 ppm of IAA concentration produced by JCS 6 is higher than that reported by Ramadhani (2007). Similar results were reported by Gravel *et al* (2007). *Trichoderma atraviride* produced the highest IAA levels (6.2 ug mL^{-1}, 9.8 ug mL^{-1} and 38,55 ug mL^{-1}) in the presence of 200 g mL^{-1} of tryptophan, tryptamine and tryptophol, respectively. Apart from the JCS 6 isolate, the results obtained in this study are lower than Subowo's (2013) results with an IAA concentration of 2.46 ppm for the genus Aspergillus isolates after five days of incubation. The results obtained in this study are higher than the results obtained by Abri (2015), which were 0.055 to 2.19 mg/L IAA concentration in the rhizosphere fungi of the Tanatoraja aromatic rice. Some isolates in this study have the low capability to produce IAA hormone, but they can be utilized as biological agents and microbial constituents for biofertilizers.

The GA_3 concentration of JCS 6 (4.20 ppm) in this study is higher compared to the study reported by Wisdawati (2020), who tested 62 rhizosphere fungi isolates that had the capability as PGPF in Taro sapphire with GA3 concentrations of 6.2, 9.8, and 3.93 ug mL^{-1} ppm to 5.05 ug mL^{-1} ppm. Moreover, Bhalla *et al* (2010) reported that F. solani isolate had the highest capability to produce GA_3 with a concentration of 2.26 ppm. Pandya *et al* (2018) obtained 19.56 ppm to 184.11 ppm of GA3 concentration from genus *Aspergillus*, which is higher than this study.

Fungi synthesize IAA as secondary metabolites under suboptimal growth conditions or when a precursor in the form of the amino acid tryptophan is available (Li *et al*, 2019). IAA and GA_3 are exogenous hormones produced

by fungi that are able to accelerate plant growth by stimulating the differentiation process in roots in forming hairy roots (Imaningsih *et al*, 2021). Larekeng *et al* (2019) stated that the higher the concentration of tryptophan added to the media, the higher the concentration of IAA produced. The differences in the hormone concentration produced are also affected by the conditions of sampling location, the microbial type, the nutritional content, incubation time, growth rate and the capability to convert L-tryptophan contained in the media. The capability of microbes to produce IAA and GA_3 hormones is influenced by incubation time, growth rate, tryptophan concentration, supernatant culture and dissolved oxygen concentration (Bose *et al*, 2013).

Beneficial microbes can be used to aid in plant growth promotion strategies. As a result, we believe it has the potential to play a role in promoting plant growth in practical applications (Jufri *et al*, 2021). Some studies about IAA and GA_3 function as plant growth regulators, bamboo in tissue culture (Gusmiaty *et al*, 2020; Larekeng *et al*, 2020), in *Zizania latifolia* (Li *et al*, 2019), *Sedum alfredii* Hance showed the results indicated that spraying IAA/GA_3 alone or in combination with OA increased plant growth (Liang *et al*, 2021).

CONCLUSION

Based on the ability assays to produce IAA and GA_3 hormones by Rhizosphere fungi isolates, the most potential rhizosphere fungus in producing phytohormones was JCS6 isolate. This research will be expanded to determine the isolate's type and function.

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REFERENCES

Abri A (2015) Isolasi cendawan rhisozfer penghasil hormone indol acetic acid (IAA) pada padi aromatik Tanatoraja. *Prosiding Seminar Nasional Biologi* **1**(1).

Arif A, Larekeng S H, Restu M, Cahyaningsih Y F and Mukti J (2019) A Genetic Diversity on Jabon Merah (*Anthocephalus macrophyllus* Roxb.) from three Different Provenances in South Sulawesi. *IOP Conference Series: Earth and Environmental Science* **270**(1). https://doi.org/10.1088/1755-1315/270/1/012003

Batti J R, Larekeng S H, Arsyad M A, Gusmiaty and Restu M (2020) *In vitro* growth response on three provenances of Jabon Merah based on auxin and cytokinin combinations. *IOP Conference Series: Earth and Environmental Science* **486**(1), 1–16. https://doi.org/10.1088/1755-1315/486/1/012088

Bhalla K, Singh S B and Agarwal R (2010) Quantitative determination of gibberellins by high-performance liquid chromatography from various gibberellins producing Fusarium strains. *Environmental Monitoring and Assessment* **167**(1), 515–520.

Borrow A, Brian P W, Chester V E, Curtis P J, Hemming H G, Henehan C, Jeffreys E G, Lloyd P B, Nixon I S, Norris G L F and Radley M (1955) Gibberellic acid, a metabolic product of the fungus Gibberella fujikuroi: Some observations on its production and isolation. *J. Sci. Food Agricult.* **6**(6), 340–348. https://doi.org/10.1002/jsfa.2740060609

Bose A, Shah D and Keharia H (2013) Production of indole-3-acetic-acid (IAA) by the white-rot fungus *Pleurotus ostreatus* under submerged condition of Jatropha seedcake. *Mycology: An International Journal on Fungal Biology* **4**(2), 103–111. https://doi.org/10.1080/21501203.2013.823891

Danu P K P and Subiakto A (2015) Pertumbuhan stek jabon merah (Anthocephalus macrophyllus (Roxb.) Havil) pada berbagai media dan zat pengatur tumbuh. *Jurnal Penelitian Hutan Tanaman* **12**(2), 123–130.

Elias F, Woyessa D and Muleta D (2016) Phosphate solubilization potential of rhizosphere fungi isolated from plants in Jimma Zone, Southwest Ethiopia. *Int. J. Microbiol.* **2016**, Article ID 5472601 | https://doi.org/10.1155/2016/5472601

Gravel V, Antoun H and Tweddell R J (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). *Soil Biology and Biochemistry* **39**(8), 1968–1977.

Gusmiaty Restu M, Larekeng S H and Setiawan E (2020) The optimization of *in vitro* micropropagation of betung bamboo (*Dendrocalamus asper* backer) by medium concentrations and plant growth regulators. *IOP Conference Series: Earth and Environmental Science* **575**(1). https://doi.org/10.1088/1755-1315/575/1/012024

Hasan H A H (2002) Gibberellin and auxin-indole production by plant root-fungi and their biosynthesis under salinity-calcium interaction. *Rostlinna Vyroba* **48**(3), 101–106. https://doi.org/10.1556/AMicr.49.2002.1.11

Hedden P (2020) The current status of research on gibberellin biosynthesis. In : *Plant and Cell Physiology* **61**(11), 1832–1849). Oxford University Press. https://doi.org/10.1093/pcp/pcaa092

Imaningsih W, Rahayu N D and Hakim S S (2021) *Endophytes and Rhizosphere Fungi from Galam (Melaleuca cajuputi Powell.) which has the Potential to Produce Indole Acetic Acid (IAA).*

Iradhatullah R, Kuswinanti T, Asrul L and Rasyid B (2015) *Growth Rate and Indole Acetic Acid Production of Several Fungal Rot Isolates* **4**(6), 2013–2015.

Jufri S W, Iswanto, Larekeng S H and Arif A (2021) Isolation and identification of fungi associated with natural forest land and post-mining areas of PT. Vale Indonesia: Preliminary study. *IOP Conference Series: Earth and Environmental Science* **807**(4). https://doi.org/10.1088/1755-1315/807/4/042073

Larekeng S H, Gusmiaty G and Nadhilla D (2020) *In-Vitro* Shoot Induction of Pring Tutul (*Bambusa maculata*) through in various Plant Growth Regulators (PGR). *IOP Conference Series: Earth and Environmental Science* **575**(1). https://doi.org/10.1088/1755-1315/575/1/012139

Larekeng S H, Gusmiaty G, Restu M, Tunggal A and Susilowati A (2019) Isolation and identification of rhizospheric fungus under Mahoni (*Swietenia mahagoni*) stands and its ability to produce IAA (Indole Acetid Acid) hormones. *IOP Conference Series: Earth and Environmental Science 343 012051*, **343**, 1–12. https:/

/doi.org/10.1088/1755-1315/343/1/012051

Larekeng S H, Restu M, Arif A, Cahyaningsih Y F and Mukti J (2019) A genetic approach to study mating system on Jabon Merah (*Anthocephalus macrophyllus* Roxb.) from three different provenances in South Sulawesi. *IOP Conference Series: Earth and Environmental Science* **235**, 012049. https://doi.org/10.1088/1755-1315/235/1/012049

Li J, Guan Y, Yuan L, Hou J, Wang C, Liu F, Yang Y, Lu Z, Chen G and Zhu S (2019) Effects of exogenous IAA in regulating photosynthetic capacity, carbohydrate metabolism and yield of *Zizania latifolia*. *Scientia Horticulturae* **253**, 276–285. https://doi.org/10.1016/J.SCIENTA.2019.04.058

Liang Y, Xiao X, Guo Z, Peng C, Zeng P and Wang X (2021) Co-application of indole-3-acetic acid/gibberellin and oxalic acid for phytoextraction of cadmium and lead with Sedum alfredii Hance from contaminated soil. *Chemosphere* **285**. https://doi.org/10.1016/j.chemosphere.2021.131420

Mompewa N M, Yunianti A D and Larekeng S H (2019) Karakteristik Struktur Anatomi Dinding Sel Jabon Merah (*Anthocephalus macrophyllus*) Provenansi Wajo. *Perennial* **15**(1). https://doi.org/10.24259/perennial.v15i1.6496

Pandya N D, Desai P V, Jadhav H P and Sayyed R Z (2018) Plant growth promoting potential of *Aspergillus* sp. NPF7, isolated from wheat rhizosphere in South Gujarat, India. *Environmental Sustainability* **1**(3), 245–252.

Ramadhani D (2007) Formulasi pupuk bioorganik campuran Trichoderma harzianum dengan kascing.

Rao N S S (2010) Mikroorganisme tanah dan pertumbuhan. Universitas Indonesia.

Tambingsila M (2016) Identifikasi dan Uji Efektivitas Cendawan Rhizosfer Tanaman Kakao sebagai Antagonis Pengendali (*Phytophthora palmivora* Bult.). Penyebab Busuk Buah Kakao. *Jurnal Agropet* **13**(1), 12–23.

Wisdawati E (2020) *Eksplorasi dan Studi Keefektifan Cendawan Pemacu Pertumbuhan Tanaman (PGPF) dan Antagonis Terhadap Penyakit Hawar Daun Pada Tanaman Talas SAFIRA*.