SCREENING OF GIBBERELLIN PRODUCING RHIZOSPHERE FUNGI

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Abstract - The larger use of pesticide and synthetic fertilizer on the agricultural land brings negative impact to environment, plant and human health. Selecting potential gibberellin producing rhizosphere fungi is assumed as an effort to reduce synthetic fertilizer use on farms. In this study, we select potential rhizosphere fungi in producing gibberellin. Produced gibberellin concentration was various, at 3,95-4,69 µg l^{-1} which isolate ETR29 appears as the most productive one. Morphological identification result showed that isolate ETR29 is Aspergillus spp

Keywords - Fungi, Giberellic, Rhizosphere.

I. INTRODUCTION

Modern agriculture does depend on chemical materials use to boost plant productivity. The effort in achieving higher productivity is carried out by adding chemical materials such as growth regulator substance, synthetic fertilizer and pesticide although it costs quite expensive and leaves residue on farms as well as agricultural product yielded. It can pollute the environment and affect plant and human health. Hence, biotechnology products started to be developed to solve those problems. One of them is the development of phytohormone gibberellin (GA3) producing rhizosphere to spur plant growth.

Rhizosphere is an ideal vicinity for the growth and development of soil microorganism since it is influenced by root exudation of plant. This microorganism forms colony around plant root, experiencing mutual activity for plant growth both directly or indirectly. Furthermore, it is able to substitute inorganic input. Interaction between plant and organism runs mutually. Plant attracts mutual microbe in rhizosphere vicinity by taking out root exudate that acts as nutrient source by microbe, in the other side, gibberellin is the produced hormone by microbe, used by plant to set biological growth process, differentiation and plant growth.

Gibberellin in the plant functions to break dormancy, promote flowering [1, 2], boost seed germination process and cell elongation [3, 4]. Gibberellin is produced by plant, fungi [5], and bacteria [6, 7]. Gibberellin producing fungi are Phoma herbarum [8], Phoma glomerata[9], Aspergillus niger [10], Fusarium moniliforme [11].

Since rhizosphere fungi plays important role in increasing gibberellin hormone availability inside the plant, consequently, it needs to isolate and identify types of fungi in the rhizosphere vicinity of taro plant living in the natural ecosystem. Hopefully, along with obtained fungi species which are able to produce gibberellin, and synthetic fertilizer use on farms can be diminished as well.

II. DETAIL EXRIMENTAL

2.1 Rhizosphere Fungi Isolation

Soil sampling adopted Composite sampling method [12]. Soil sample was taken from 10 sampling points randomly at 10- 15 cm depth from the soil sticking at plant root hairs. 10 grams of composited soil were suspended in 100 ml aquades. Subsequently, 1 ml suspense was fed into 9 ml sterile aquades inside the reaction tube then stirred until homogenous (dilution 10^{-1}), the same thing done until dilution 10^{-6}. The dilution result at 10^{-1} -. 10^{-6} , were put 1 ml respectively into petri dish filled with PDA medium (Potato Dextrosa Agar) and making them even by spatula. Then, incubated at room temperature for 2-3 days. Growing fungi colonies at dilution 10^{-1} -10^{-6} were purified by moving one fungi colony to new sterile PDA medium then analyzed to detect the isolation capability in producing gibberellin.

2.2 Gibberellin (GA3) Production.

Gibberellin acid production by microorganism is determined by following [13]. Prepare sterile 100 ml Potato Dextrosa Broth (PDB) then add mycelium of fungi isolate and incubated at 37ºC for seven days. After 7 days incubation, culture filtrate was centrifuged at 8000 g for 10 minutes to dispel fungi cells. Move 15 ml of the culture into reaction tube and add 2 ml zinc acetate solution. After 2 minutes, add 2 ml potassium ferrocyanide solution and centrifuged at 8000 g for 10 minutes. Move 5 ml supernatan to reaction tube and add 5 ml chloride acid 30%, get them incubated at 27ºC for 75 minutes. Blanko was prepared with chloride acid 5% and absorbance measured 254 nm in spectrophotometer.

2.3. Identification

Fungal isolates from rhizosphere were identified macroscopically by observing their colony morphologies including texture, color, reverse color, and radial and concentric lines. The microscopic

identification was performed by viewing the hypa, sporangium, conidiophore, and conidia [14].

III. RESULT AND DISCUSSION

The measurement result of rhizosphere fungi capability in producing gibberellin through calorimetry demonstrated that production of GA3 by 10 isolates of rhizosphere fungi was really diverse ranging from 3,95-4,69 μgl^{-1} (fig 1).

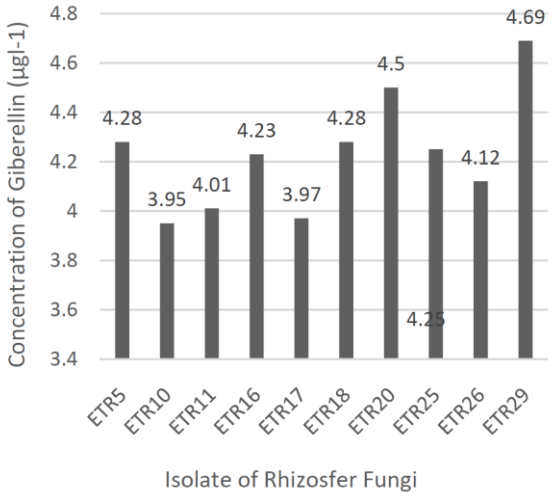


Fig 1. Giberellin production of rhizosfer fungi

Isolate ETR29 owns the highest gibberellin production compared to the others, it is 4,69 μgl^{-1}. Commonly, isolate has capability to produce gibberellin above 4 μgl^{-1} only ETR17 and ETR10 which product gibberellin < 4 μgl^{-1} . The research conducted by [8]stated that Phoma herbarumcould producegibberellin (GA3) , 2,9 ng/ml and [9] found fungus Phoma glomerata could produce gibberellin 4,34 ng/ml. Gibberellin measurement through calorimetry, applied method from [13] using zinc acetate and potassium ferrocyanida reactors. [15] and [16] have experimented the same study.

Microorganism such as bacteria and fungi associated plant roots could promote growth and development of plant through generating secondary metabolite, for instance, plant hormone of gibberellin (GA3) [17, 6]. Research conducted by Gusmiaty[18] showed that bacteria are able to Produce gibberellin as well. The test result of 35 isolates bacteria indicated that produced gibberellin was around 1,959-4,322 μgl^{-1}. The same research has been carried out by Kafrawi [19] by isolating rhizobacteria in the vicinity of onion rhizosphere, finding isolate bacteria could produce gibberellin at 1,254-1,376 μgl^{-1}.

Gibberellin hormone is categorized in diterpenoid class , controlling many aspects of plant life include growth process and plant development [20, 21, 22], it takes role in germination, stem elongation, meristematic tissue development and plant organ differentiation [23], but mostly to promote growth or stem elongation.

Fungi isolate ETR29 is an isolate with highest gibberellin production among ten fungi found, hence, isolate ETR 29 requires identification and characterization. Morphological identification result by carrying macroscopic characterization isolate has green colony centre with white edge colony. Its reverse colony is white while in microscopic characterization, isolate appears to have long and smooth stipe, vesicle, metula, conidiophore and smooth globose conidia (fig. 2).



Fig 2. Macroscopic and microscopic isolate ETR29

Thus, based on macroscopic and microscopic nature, isolate ETR29 is considered as Aspergilus spp. According to [24] and [25], Aspergillus spp has head structure, stipe, vesicle, metulae, phialide, conidia, ascomata dan ascospores. Research of Bilkay et al [10] informed that Aspergillus niger type could produce GA 238,7 μgl^{-1} which incubated for 10-16 days.

IV. CONCLUSIONS

Gibberellin production from rhizosphere fungi around 3,95-4,69 μgl^{-1} with highest gibberellin production is at isolate ETR29. Morphological observation showed isolate ETR29 is Aspergillus spp, It is potential to be developed as exogenous growth regulator substance and organic fertilizer.

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