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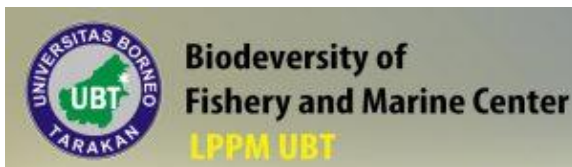
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CABE 2021

International Conference on Climate Change, Agriculture, Biodiversity, and Environment Study (CABE 2021)

Tarakan, North Kalimantan – Indonesia, 23-24 December 2021

Organized By:



Preface

International Conference on Climate Change, Agriculture, Biodiversity, and Environment Study (CABE 2021) was held in Tarakan, Indonesia. CABE 2021 was hosted by Biodiversity of Fishery and Marine Center LPPM UBT.

The conference is organized by the Research and Community Services Center of Borneo Tarakan University, Indonesia. The primary objective of The CABE 2021 is to promote effective interaction and cooperation among scientists and technicians who are involved in agriculture research and development in the world with the view of encouraging and facilitating research activity, implementing research findings, sharing of information and publication of research results. The CABE 2021 focuses on both theory, design and applications. In addition to the technical sessions, there will be invited sessions, panel sessions and keynote addresses.

At the moment, we are facing a new situation that has never happened before, the Global Pandemic caused by Coronavirus Disease of 2019 (Covid-2019). This issue has affected the lives of people globally, Including the lives of academics in education. The Covid-19 pandemic is an unprecedented phenomenon for us all. The situation is continually evolving, and we must face new challenges every day. With the appeal above, the International conference on Conference on Climate Change, Agriculture, Biodiversity, and Environment Study has been switch into virtually mode. Originally the coference was planned in a physical conference. However, until mid-September 2021, the conditions for Covid-19 were not normal. The participants really need the publication results as an annual performance report. In this case, all participants refuse if the conference is postponed. At 23-24 December 2021, all participants were invited virtually for preparation and simulation. In the conference day, all committee were organizing the conference in virtually using zoom application from Tarakan, Kalimantan, Indonesia. The structure were similar with the physical conference as indicated in the following conference program. The keynote speakers session was cunducted in the morning and continued with parallel sessions after lunch break. In the parallel session, each participant was preset their paper for 15 minutes including questions and answers. The CABE 2021 were attended around 170 audience with 121 presenters from academicians, students, scientists, and other related professionals.

Our special thank also goes to all individuals and organizations such as the international program committees (IPC), the conference organizers, the reviewers, and the authors, for their contribution in making CABE 2021 not only a successful international conference but also as a memorable gathering event. We are also grateful for the support of the publication service of IOP. We hope that it should give you a beautiful memory to bring home in addition to new insights and friends gathered during the conference. We are truly grateful for your contribution and interest. We hope that you will get pleasure from CABE 2021 in this beautiful city, Tarakan, Indonesia.

Best regards,

Dr. Ratno Achyani, S.Pi, M.Si (General Chair of CABE 2021)

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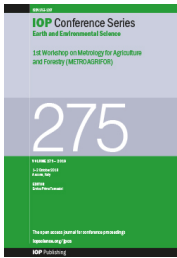
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- **Number of submissions accepted:** 121
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The Effect of *Beijerinckia fluminensis* G3 and *Rhizobium pusense* G4c on Germination of rice Var. Ciherang and red rice Var. Barak Cenana

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Abstract. The productivity of rice fields in Indonesia is decreasing and production per unit area is also decreasing due to the shifting of functions from agricultural land to non-agricultural land. Excessive land continuous use of chemical fertilizers can damage the soil so the solution offered is the use of biological fertilizers, namely by utilizing the use of microorganisms from nature. This study aims to determine the effectiveness of bacteria G3 and G4c that have been obtained by previous researchers against root weight, root length, root width, root volume, root surface area in rice Var. Ciherang and red rice Var. Barak Cenana. Making NFb media as a selection medium then propagated on TSA media and a confirmation test is carried out. The results showed treatment with G3 (*Beijerinckia fluminensis*) on rice Var. Ciherang had the highest average root weight, average root length, root width, root volume and root surface area compared to the control and G4c (*Rhizobium pusense*) treatment. Control treatment without giving G3 (*Beijerinckia fluminensis*) and G4c (*Rhizobium pusense*) on red rice Var. Barak Cenana had the highest average root weight, average root length, root width, root volume and root surface area compared to G3 (*Beijerinckia fluminensis*) and G4c (*Rhizobium pusense*).

Keywords: *Beijerinckia fluminensis* G3, *Rhizobium pusense* G4c, rice Var. Ciherang, red rice Var. Barak Cenana.

1. Introduction

Indonesia, China, India, Bangladesh, Vietnam, Japan, Thailand, Myanmar and Pakistan are rice producing countries and use rice as the main energy source [1]. Rice is a basic food that contributes 35-60% of the calories consumed by Asians [2]. Rice production in Indonesia has not yet met consumer demand due to increasing population growth and reduced land so that the Indonesian government has to import rice from abroad based on FAO. The productivity of rice fields in Indonesia is decreasing and production per unit area is also decreasing due to the shifting of functions from agricultural land to non-agricultural land, for example for development. This can be proven in 2018 the area of raw rice fields was only 7.1 million hectares, down compared to 2017 which was still 7.75 million hectares based on Central Bureau of Statistics in 2018. In addition, excessive and continuous use of chemical fertilizers without being balanced with organic fertilizers, apart from being inefficient,



can also damage the soil, thereby reducing soil fertility, fertilization efficiency, and reducing soil organic matter [3]. Rice plant was chosen because it is one of the important food crop commodities in Indonesia, very responsive to changes in the main nutrients, especially nitrogen [4]. Rice plant Var. Ciherang was chosen because the variety is resistant to bacterial leaf blight, high productivity, quality and taste of fluffier rice [5]. Research by [6] where the utilization of microbes such as *Beijerinckia sp.*, *Rhizobium sp.* and others are good to be developed as biological fertilizers for rice plants and the isolate got from previous research [7] that show *Beijerinckia sp.* and *Rhizobium sp.* were isolated from rhizosphere area of Barak Cenana. Based on research by [8], biological fertilizers or biofertilizers have an impact on the growth and productivity of rice plants. In conducted study by [7], isolates coded G3 (*Beijerinckia fluminensis*) and G4c (*Rhizobium pusense*) have the ability to produce IAA hormones, siderophores and phosphate solubilization. Research by [9] reported that *Beijerinckia sp.* had a significant effect on nutrient uptake by sugarcane grown on ultisols. Research by [10] stated that the inoculation of *Rhizobium sp.* can significantly stimulate the increase in plant height so that it does not need to be given more nitrogen nutrients from fertilizers.

One such approach is the utilization of inoculants composed of plant-growth-promoting bacteria (PGPR), which comprise a group of beneficial soil bacteria that associate with plants, contributing to the overall fitness of the crops, water and nutrient uptake, improving for the root development, and tolerance to biotic and abiotic stresses [11,12]. Bio-priming is an efficient strategy for introducing beneficial microbial inocula into the rhizosphere or soil [13,14]. Bio-priming is a biological entity-based seed treatment that involves hydrating seeds and inoculating them with helpful microbes. This method the great majority of the studies have been performed are using seed inoculation with soaking the seeds (hardiansyah) before sowing with a suspension *Beijerinckia fluminensis* [15,16,17,18] or *Rhizobium pusense* [19,20,21]. In implanted pastures, there is also the limitation of inoculant application, another method would be via leaf-spray it is worth mentioning that there are positive indications of success of leaf-spray of *A. brasilense* in maize [22,23], encouraging to verify the feasibility on pastures but via leaf spray it will works on some specific part such as the leaf.

The Efforts to use Plant Growth Promoting Rhizobacteria (PGPR) such as *Beijerinckia sp.* and *Rhizobium sp.* which has proven its ability in vitro is expected to be used for specific species such as rice and environmentally friendly biological fertilizers. The next application is its utilization in an effort to improve the condition of land that is no longer productive or less productive into productive land for agricultural businesses. This study aims to test the effectiveness of bacterial isolates coded G3 (*Beijerinckia fluminensis*) and G4c (*Rhizobium pusense*) which can produce the Indole Acetic Acid hormone obtained by [7] on increasing rice germination parameters Var. Ciherang and red rice Var. The Barak Cenana is also expected in the future to be able to grow plants that are healthier, free from pests and diseases, high production, environmentally friendly, sustainable and can reduce inorganic fertilizers so as to increase soil fertility and increase soil organic matter.

2. Material and methods

2.1. Research location

The research was conducted at the Microorganism Biotechnology Laboratory and Microorganism Biotechnology Laboratory, Faculty of Technobiology, Surabaya University.

2.2. Tools

The tools that will be used in this study include glassware beaker (Pyrex), test tube (Pyrex), screw tube (Pyrex), Petri dish (Anumbra), analytical balance (OHAUS), micropipette 10-100 L (BioRad), micropipette 100- 1000 L (Gilson), measuring cup (Iwaki), ruler (Butterfly), spectrophotometer (genesys 105 Uv-Vis), oven (memmert & binder), Falcon tube, microscope (OptiLab & Olympus), Laminar Air Flow (LAF), vortex (Faithfull), autoclave (Hirayama), hotplate stirrer (Thermo), microcentrifuge (Thermo), shaker incubator (Innova 40 & Finder), colony counter (H-EBCC), pH

meter (Metler toledo), Duran desiccator, round loop, needle loop, glass stirrer, scissors, mortar, culture bottle.

2.3. Materials

The materials used in this study include pure culture bacteria G3 and G4C, tip 10-100 L (GenFollower), tip 100-1000 L (GenFollower), glycerol (Merck), HCl (Merck), FeCl₃ (Merck), Indole Acetic Acid (Merck), Tryptic Soy Agar (Merck), Tryptic Soy Broth (Merck), Nutrient Agar (HiMedia), Plate Count Agar (Merck), lysol, Media Sulfide Indole Motility (Merck), kovac's reagent (Merck), Voges-Proskauer (Merck) Methyl Red Media, Urea solution (Merck), H₂O₂ indicator (Merck), barrite A and barrite B indicator (Merck), lugol (Merck), KOH (Merck), spirits, gentian violet carbol, 96% alcohol, fuchsin, filter paper sheets, distilled water, Bayclin, newsprint, 100 rice seed Var. Ciherang and red rice Var. Barak Cenana.

2.4. Rice Seed Preparation

The rice seeds are first peeled, then rinsed and washed with running water three times. Then the surface was sterilized using 70% ethanol in LAF for 1 minute. After that, the sterilized rice seeds were rinsed using sterile distilled water three times. Then proceed with sterilization using 25% bayclin solution and 3 drops of tween 20 solution and then shaken for 30 minutes. After that, the sterilized rice seeds were rinsed using sterile distilled water three times. Then proceed with sterilization using a 15% Bayclin solution and shake it for 15 minutes. After that, the sterilized rice seeds were rinsed using sterile distilled water.

2.5. Preparation of PGPR bacteria

Bacteria coded G3 (*Beijerinckia fluminensis*) and G4c (*Rhizobium pusense*) were grown on TSB media [54]. The cells will be shaken until they reach the exponential phase and will be inoculated for 30 minutes on sterilized rice [55]. As for the control treatment, the rice seeds will be soaked in sterile water for 30 minutes. One hundred seeds inoculated in each treatment and control were put into sterile petri dishes containing twenty-five filter paper per petri dish and the Petri dishes were stored in an incubator at 27°C for 120 hours.

2.6. Germination Parameters

Calculation of germination parameters on day 5 which includes Root Weight [56], Root Length [56], Root Width [56], Root Volume [57], Root Surface Area [56] are done one by one. Data analysis techniques used is hypothesis testing analysis of variance (ANOVA) at an error rate of 5% and will be further tested with the Tukey test (HSD) if the analysis of variance shows a significant difference [58].

3. Result and discussion

3.1. Bacteria Confirmation Test

Bacterial isolates G3 (*Beijerinckia fluminensis*) and G4c (*Rhizobium pusense*) were grown on NFb media, it was hoped that bacteria had the ability to fix nitrogen so that microorganisms that did not have the ability to fix nitrogen could not survive in this medium, then colony selection and bacterial purification were carried out until a single colony was obtained.

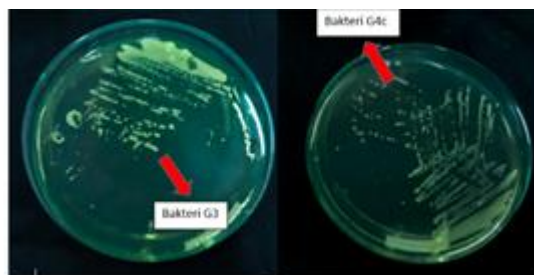


Figure 1. (a) G3 bacteria, (b) G4c bacteria

Based on Figure 1 and Figure 2 colony morphology G3 and colony morphology G4c on NFb and TSA media obtained are in accordance with the results of research [7].

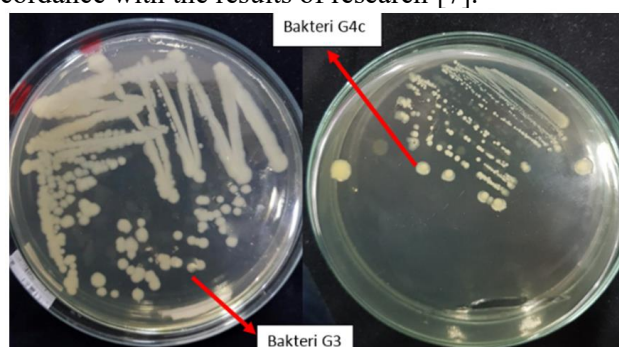


Figure 2. (a) G3 bacteria, (b) G4c bacteria

Based on the morphological characteristics obtained, isolate G3 had morphological similarities with *Beijerinckia fluminensis* and isolate G4c had morphological similarities with *Rhizobium pusense*. This is supported by research by some studies [24, 25, 26, 27] which stated that the characteristics of *Beijerinckia fluminensis* were small, white, convex, round, flat edges and Gram staining showed negative results, stems, small while *Rhizobium pusense* was Colonies were white, round, not transparent, convex, flat edges and Gram staining results were negative.

3.2. Bacterial Biochemical Test

The biochemical activity test was carried out to determine the ability of bacteria to use macromolecules and micromolecules to produce energy for bacteria and to determine the ability to synthesize certain enzymes [28]. Based on biochemical tests, isolates G3 and G4c can produce indole which can act as a signaling molecule between bacteria and plants [29, 30, 31].

Table 1. Biochemical test on isolates of bacteria G3 (*Beijerinckia fluminensis*) and G4c (*Rhizobium pusense*).

Characteristics	G3 (<i>Beijerinckia fluminensis</i>)	G4c (<i>Rhizobium pusense</i>)
Motility	+	+
Indole	+	+
Voges Proskauer	+	+
Urease	+	+
Katalase	+	+
Motility	+	+

Indole	+	+
Voges Proskauer	+	+
Urease	+	+

Research conducted by some studies [29, 30] stated that the indole hormone produced by bacteria can increase the overall root biomass, thus enabling plants to absorb water and minerals better. Isolates G3 and G4c have motile properties that can affect mobility in the soil [32] so that they can easily colonize roots. This is supported by the research [33, 34, 35, 36] strongly suggest that bacterial motility has an important role in bacterial mobility. G3 and G4c isolates can produce nonacidic or neutral end products, such as acetylmethylcarbinol and produce catalase enzymes that can protect plants from disease. The same thing has been reported by some studies [32, 37, 38]. Isolates G3 and G4c showed positive results for urea in which all isolates were able to fix nitrogen and also convert nitrogen into ammonia which can be used directly by plants [39].

3.3. Relative Value of Root Weight, Root Length, and Root Width

Based on the results shown in Figure 3 and Figure 4, the average relative values of root weight, root length and root width of G3 and G4c treatments had higher yields when compared to the control in Var rice. Ciherang, on the other hand, the G3 and G4c treatments produced relatively lower values compared to the control in red rice. Var Barak Cenana.

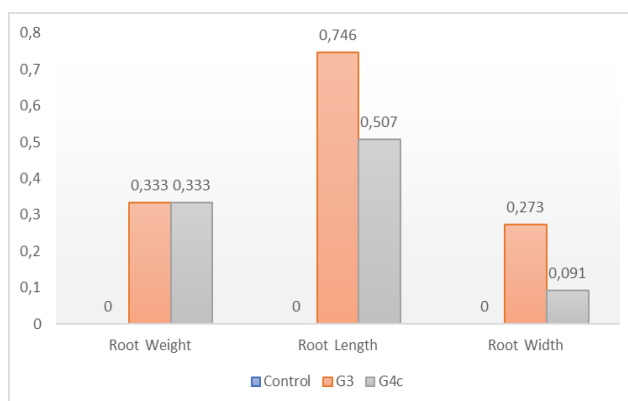


Figure 3. The graph of the average relatives value of root weight, root length and root width against the control of rice Var. Ciherang



Figure 4. The graph of the average relatives value of root weight, root length and root width against the control of red rice Var. Barak Cenana

The results of the highest IAA concentration in this study were not as large as those obtained by Lina [32] research of 40.59 ppm and Riyanti [40] which was 100 ppm but the final result still had a significant impact on root weight, root length and root width in rice. Var Ciherang. This shows the need for IAA hormone concentrations in each tissue at various stages and the response of each plant species is different [42]. Research by [43, 44, 45] also reports related matters. In addition, the association of endophytic bacteria with their host must have appropriate specificity [46, 47]. Research conducted by some studies [48, 49, 50, 51] stated that not all endophytic bacterial strains have positive interactions with their host plants [45]. Also research by studies [52, 53] stated that the length of soaking the seeds had a significant effect on rice parameters.

3.4. Root Volume and Root Surface Area Test

Based on the result Figure 5 and Figure 6, G3 treatment on rice Var. Ciherang got the highest yield at root volume and root surface area.

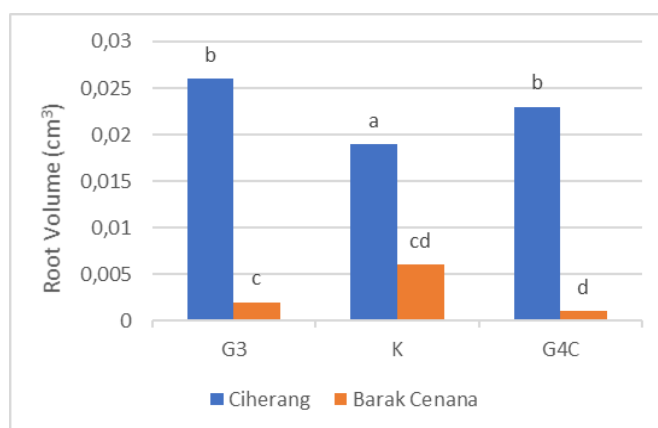


Figure 5. Root Volume diagram for rice Var. Ciherang and red rice Var. Barak Cenana

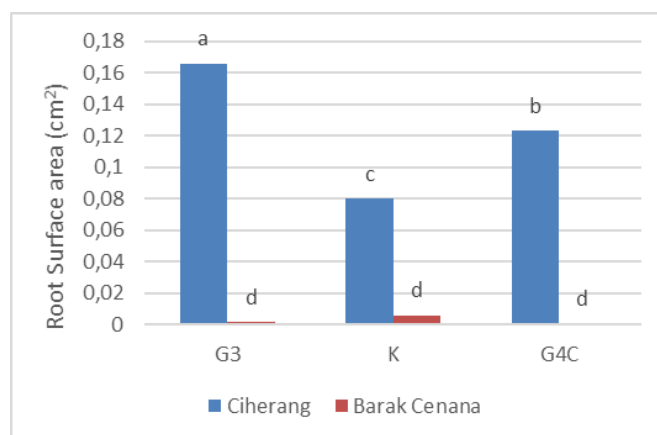


Figure 6. Root Surface Area diagram for rice Var. Ciherang and red rice Var. Barak Cenana

IAA produced by isolates G3 and G4c will be utilized by plants and will undergo metabolic processes in the plant body so that it helps in the process of increasing height and root length of plants [31]. Rice root weight Var. Ciherang was high in G3 and G4c treatments due to an increase in water

uptake by plant cells [31]. Root length also affects the absorption of water and nutrients from the environment so that it affects root weight. Plants with large root volumes are able to absorb more water so that they can survive in conditions of water shortage [53].

4. Conclusion

Treatment with *Beijerinckia fluminensis* G3 on rice Var. Ciherang had the highest mean of root weight, average root length, average root width, root volume and surface area which were 0.061 ± 0.021 g; 0.044 ± 0.016 g; $3,510 \pm 1,533$ cm; 0.138 ± 0.054 cm; 0.026 cm³; and 0.166 cm² while the control treatment for red rice was Var. Barak Cenana had the highest mean root weight, average root length, average root width, root volume and root surface area which were 0.048 ± 0.014 g; 0.008 ± 0.013 g; 0.194 ± 0.317 cm; 0.053 ± 0.091 cm; 0.006 cm³; and 0.006 cm². So, *Beijerinckia fluminensis* G3 have the potential to increase the yield of rice productivity Var. Ciherang.

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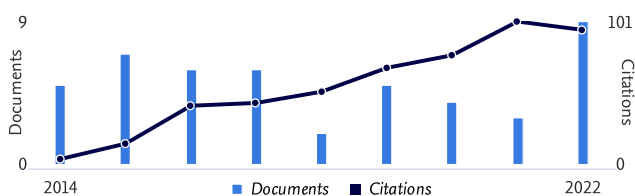
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