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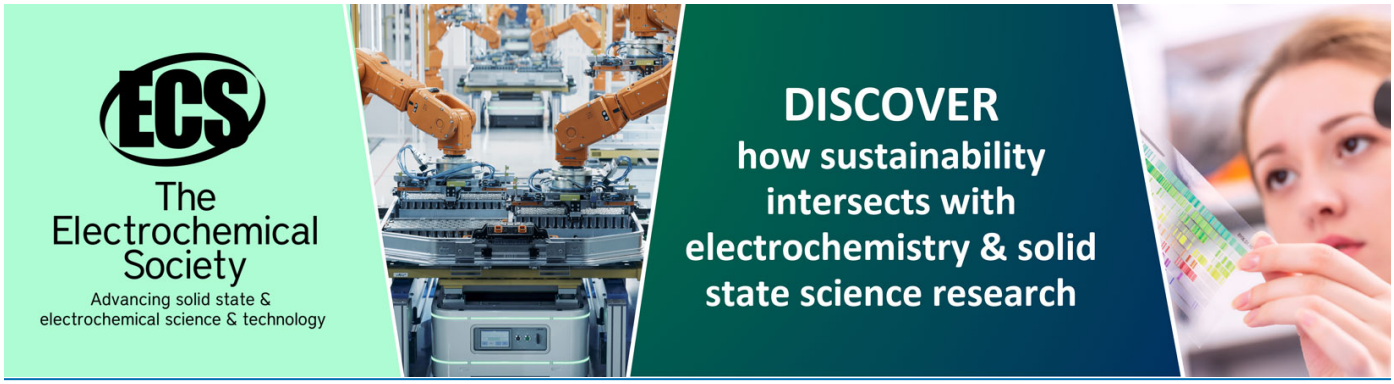

Effect of the indigenous *trichoderma* application on germination of black glutinous rice seed

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Abstract. Trichoderma is one of the rhizosphere microorganisms that are able to play as plant growth promoting fungus (PGPF) because it can produce IAA hormone. The ability of Trichoderma as PGPF has been extensively studied, but the indigenous Trichoderma effect derived from the rhizosphere of rice crops on local rice variety of west Sumatera has not been widely studied. This study aims to determine the effect of *Trichoderma* application on black glutinous rice seed germination. The experiment is done by soaking the local black sticky rice seeds with *Trichoderma* spore suspension with 10^7 spore / mL density for 24 hours. The germination rate of each isolate observed at seven days after sowing. The results showed that the type of isolate affected the rate of germination of rice seeds. The highest germination was found in isolates from the rhizosphere of rice varieties cisokan balang (94%) and the lowest was in the control (74%).

1. Backround

Rice is also a staple food for the Indonesian population, as well as ingredients to be processed into various types of supplementary food. Rice used for staple food is characterized by high amylose content. In contrast, rice with low amylose content is fluffy known as glutinous rice. Based on the color, rice consists of three groups, namely white rice, brown rice, and black rice. The colors of red, purple and black pigments are found in the pericarp layer to the outer layer of rice endosperm [1]. This color is caused by the cells in the rice epidermis containing anthocyanin [2]. Anthocyanin is a component of flavonoids, namely polyphenol derivatives in plants that have the ability as antioxidants, anticancer and prevent coronary heart disease by preventing narrowing of arteries [3]

The demand for black sticky rice is increasing with increasing population and various forms of processed food. Black sticky rice is usually used as a basic material to make snacks, such as brownies, black sticky rice tape, rengginang, black sticky rice porridge, and other traditional foods [4]. Generally black sticky rice which is widely planted by farmers is black glutinous rice of local varieties [5].

The local variety is still preserved due to the tastes that are better than superior varieties. Even so, local varieties generally have a long enough life (5-6 months) and a potential yield of 40-50% lower than superior varieties [5]. In addition, the seeds of local varieties that are used by farmers are of low quality because they are obtained from crops previously and inherited from generation to generation [6]. Only 5% of farmers use quality and labeled seeds for lowland rice farming in West Sumatera [7]

Long plant life of local varieties is due to low growth rates. If this variety is used for organic farming, it is necessary to increase its growth rate. This is because, in organic farming the Rice Intensification System (SRI), the age of the plant moves to the field at the age of 10 days with the number of plants of one seed per hill. If slow plant growth results in very sensitive to biotic environmental stresses and abiotic

Increased plant growth can be done by giving synthetic hormones or by utilizing microbial potential, especially those classified as Plant Growth Promoting Fungi (PGPF). One mushroom that is widely used as PGPF is a fungus from the genus *Trichoderma*. *Trichoderma* is a soil saprophytic fungus that is antagonistic to pathogenic fungi in plants [8] and is able to produce Indole Acetic Acid (IAA) hormones. *T. harzianum* is able to produce IAA hormone which is produced optimally on the 3rd day of incubation which plays a role in plant growth [9]. Significant effects on seed germination and seedling vigor were also observed in the seeds inoculated by *Trichoderma* spores [10]. Germination of Chile increases with Trichoderma application. Among the five *Trichoderma* strains, *T. harzianum* IMI 392432 culture filtrate exhibited a significant percentage of germination percentage in chili seeds both in laboratory and field conditions followed by *T. harzianum* IMI 392433, *T. harzianum* IMI 392434, *T. virens* IMI 392430 and *T. pseudokoningii* IMI 392431 [11].

Trichoderma sp. SL2 which is formulated with corn, can significantly increase the growth of rice seeds [12]. Germination rate and vigor index of rice seeds given *Trichoderma sp*. higher than control [13].. The application of *T.harzianum* can increase rice seed germination [14]. *Trichoderma asprellum* SL2 can significantly increase root length, wet weight and biomass of rice plants compared to controls [15]. The application of *T. harzianum* WKY1 as a dual purpose bio-agent for biological control of anthracnose disease and plant growth promotion [16]. On the contrary, it was also found that *Trichoderma* had no effect on plant growth. Application of 3 *Trichoderma* isolates originating from 3 provinces of corn cultivation in Sulawesi did not affect the on seed corn germination ([17]. Tomato seed germination rate was not affected by *Trichoderma* application [18]. The inconsistent influence of *Trichoderma* on increasing plant growth, then this study is to evaluate some indigenous *Trichoderma* isolates on rice seed germination

2. Materials and Methods

The study was carried out from February to November 2017 at the Microbiology and Screenhouse Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang. The *Trichoderma* isolates selected for this study were obtained from the soil of paddy field in Solok district West Sumatera Province, Indonesia. Trichoderma colonies grown on the medium were transferred to a new PDA medium. Displacement is done by taking the mycelium sample with the corkborer. Subsequently incubated at room temperature until the Trichoderma mycelium grew to meet the petri dish. *Trichoderma* which has been purely propagated for the manufacture of suspensions. Trichoderma suspension is made by harvesting molded spores with dark green spores. 10 mL sterile distilled water is poured into a petri dish containing *Trichoderma* until the spore release from the mycelium. The solution containing *Trichoderma* spores was transferred into a new test tube and the spore density was calculated with a density of 107 spores/mL. Rice seeds are chosen for research that is nourishing. Black glutinous rice seeds are soaked in backer glass containing water. Floating seeds are taken and discarded, while the sinking seeds are taken for use in research [19]. Before being treated, the seed surface is sterilized by immersing in 70% alcohol for 30 seconds, in 1% sodium hypochlorite for 1 minute, then the seeds are rinsed twice with sterile water [20]. The treatment was carried out by taking fifty grain of rice seeds to be soaked in each treatment consisting of 5 isolates and one control. Immersion was carried out in a test tube containing 10 mL of *Trichoderma* spore suspension with a density of 107 spores / mL for 24 hours. In the control, the seeds are soaked in a test tube containing 10 mL of distilled water with the same duration of time as other treatments. After being spawned for 2 days, the seeds are transferred into the nursery tray containing the soil that has been immobilized. Observations were made on the level of seed germination from

each treatment given. Observations were made at the age of 7 days after sowing. Seed germination rate (GR) can be calculated using the following formula;
GR = (number of seeds that germinate) / (the number of seeds in the seedling tray) × 100% [21].

3. Result and Discussion

The results showed that Trichoderma application was able to increase black glutinous rice seed germination. Seed germination rates ranged from 74% -94%. The lowest percentage of germination rate was found in the control which was 74% and followed by the treatment of *T. asprellum* SL2 (ASP) with a percentage of 75%. While the percentage of the highest germination rates found in SB and RE isolates were 94% and 91% as shown in the figure 1.

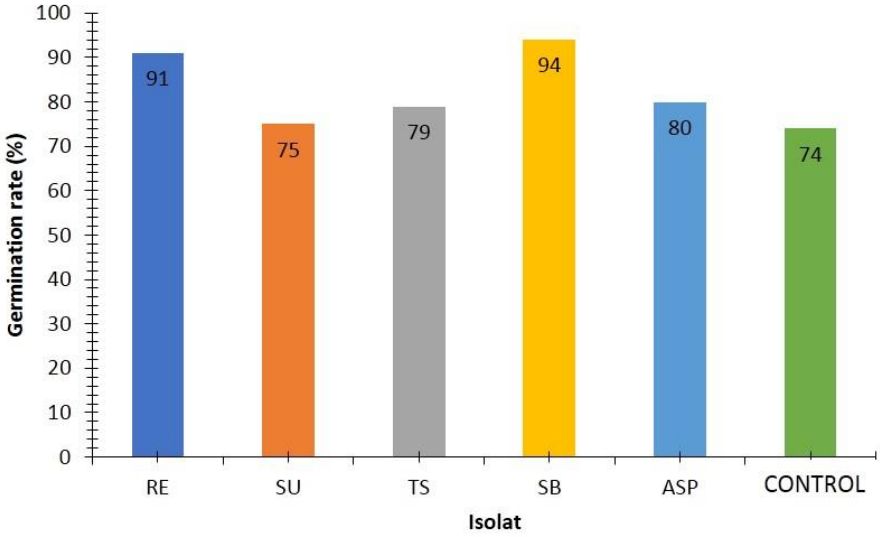

Figure 1. Germination Rate of Black Rice Sticky Seedling

Although the isolate response to germination varies, but based on the seed quality category, two isolates, SU and TS do not qualify as good quality seeds because the germination rate is below 80%. Rice seeds are said to be good if the germination percentage is 80% or more ([22]. The difference in percentage of glutinous rice seed germination is thought to be due to the potential difference of each isolate to produce phytohormone compounds such as the hormone IAA (Indol Acetate Acid). These auxin-like hormones play a role in increasing the activity of A-amylase. Amylase has a central role in the mobilization of starch and a decisive role in seedling growth [23]. It is well known that IAA is the most naturally occurring auxin in vascular plants, and it has great importance during lateral and adventurous roots initiation and emergence, and shoot development [24]

Differences in seed responses to isolates have also been reported. 101 isolates of *Trichoderma* from Colombia, and assessed the relationship of these factors to the enhancement of early stages of growth on bean seedlings. Sixty percent of isolates produced indole-3-acetic acid (IAA) or auxin analogues. The production of any of these metabolites was a characteristic of specific strains, as the ability to produce these metabolites varied greatly within species [25]. Seven *Trichoderma* isolates significantly improved the growth of bean seedlings. However, metabolite production varied widely in these seven strains, and some isolates did not produce any of the assessed growth-promoting metabolites.[25]. Growth promotion by *Trichoderma spp* depends on the strain used [26]. Plant growth promoting potential varies among different *Trichoderma* species and strains [27]

Based on the results of the study, it was found that SB and RE isolates were superior in increasing germination in local varieties of black glutinous rice compared with *T. asprellum* SL2 (ASP) isolates. Whereas it is known that ASP is the best local isolate in Malaysia in increasing the germination of

varieties of MRQ74 rice seeds [13]. These results indicate that local isolates from different regions have different effects on germination. Furthermore, it can also be understood that the response of varieties to types of isolates is also different. Seven *Trichoderma* isolates significantly improved the growth of bean seedlings. However, metabolite production varied widely in these seven strains, and some isolates did not produce any of the assessed growth-promoting metabolites [25].

4. Conclusion

Based on the research conducted, it was concluded that the application of Trichoderma had a different effect on the germination rate of black glutinous rice seeds. Three isolates were able to increase germination of rice seeds to reach the standard of quality seeds. Conversely, the germination rate of the two isolates is still below the standard criteria for quality seeds

References

[1] Abdullah, B. 2017. Increased Anthocyanin Content of Red Rice and Black Rice Through Biofortification. *Litbang Pert*. Vol. 36 No. 2 December 2017: 91-98

[2] Nailufar A. A., Basito, C.Anam. 2012. Characteristics Study of Black Glutinous Rice (*Oryza Sativa* Glutinosa) on Several Packaging Types during Storage. *Jurnal Teknosains Pangan.*1(1)

[3] Wang, G., B. Parpia, and Z. Wen. 1997. The composition of Chinese foods. Institute of Nutrition and Food Hygiene Chinese Academy of Preventive Medicine. Washington DC: ILSI Press

[4] Suhartatik, N., 1, M. Karyantina, M. N. Cahyanto, S. Raharjo, E. S.Rahayu. 2014. Fermentative Characteristics of Medium deMann Rogosa Sharpe (Mrs.) Anthocyanin Black Glutinous Rice (*Oryza Sativa* Var. Glutinosa) Using *Pediococcus Pentosaceus* N11.16. *Agritech.* 34(3).

[5] Santika, A., dan Rozakurniati. 2010. Techniques of Quality Evaluation of Glutinous Rice and Red Rice on Some Upland Rice Strains. *Buletin Teknik Pertanian*. 15(1) : 1-5

[6] *West Sumatera Assessment Institute for Agricultural Technology . 2009.* Characteristics of Specific Local Rice Varieties in Solok Regency.

[7] Nurnayetti, M. Ali, Asyiardi, Aryunis, Yunasri, dan Y. Renor. 2008. Assessment of the Dynamics of Rural Development Indicators in West Sumatra. Final Report of AIAT West Sumatera

[8] Berlian, I., B. Setyawan, H. Hadi. 2013. Antagonism Mechanism of Trichoderma Spp. Against Some Soil Contagious Pathogens. *Warta Perkaretan.* 32(2): 74 – 82.

[9] Ramadhani, D. 2007. Formulation of Mixed Bioorganic Fertilizers *Trichoderma Harzianum* with Casing. Essay. Bogor Agricultural Institute.

[10] Hajieghrari, M. Mohammadi. 2016. Growth promoting activity of indigenous Trichoderma isolates on wheat seed germination, seedling growth and yield. *AJCS* 10(9): 1339-1347

[11] Rahman, M.H., R. Sultana, M. Begum and M. F. Alam. 2012. . Effect of culture filtrates of Trichoderma on seed germination and seedling growth in chili. *Int. J.Biosci.* Vol. 2, No. 4, p. 46-55`

[12] Doni, F., A. Isahak, C. R. C. M. Zain, S. M. Ariffin, W. N. W. Mohamad, and W. M. W. Yusoff. 2014. Formulation Of Trichoderma Sp. SL2 Inoculants Using Different Carriers For Soil Treatment In Rice Seedling Growth. *SpringerPlus*. 3:532.

[13] Doni, F., I. Anizan, C.M.Z.C. Radziah, A. H. Salman, M. H. Rodzihan, and W.M. W. Yusoff. 2014. Enhancement Of Rice Seed Germination And Vigour By *Trichoderma* Spp. *Research Journal Of Applied Sciences, Engineering And Technology.* 7(21): 4547-4552

[14] Khan, A.A., A.P. Sinha and Y.P.S. Rathi. 2005. Plant Growfh Promoting Activity of *Trichoderma Harzianum*On Rice Seed Germination and Seedling Vigour. *Indian* J. *Agric. Res ..* 39 (4) : 256- 262.

[15] Doni, F., C. R. C. M. Zain, A. Isahak, F. Fathurrahman, N. Sulaiman, N. Uphoff, W. M. W.

Yusoff. 2016. Relationships Observed Between *Trichoderma* Inoculation and Characteristics Of Rice Grown Under System Of RiceIntensification (SRI) Vs. Conventional Methods Of Cultivation. *Springer. Publish Online : 08 July 2016*

[16] Wesam I. A. Saber, Khalid M. Ghoneem, Younes M. Rashad & Abdulaziz A. Al-Askar (2017) *Trichoderma Harzianum* WKY1: an indole acetic acid producer for growth improvement and anthracnose disease control in sorghum, Biocontrol Science and Technology, 27:5, 654-676

[17] Mantja, K., Y. Musa, A.Ala and A. Rosmana. 2015. Indigenous *Trichoderma* Isolated from Maize Rhizosphere with Potential for Enhancing Seedling Growth. IJSR. Vol 4 (6):1814-1818

[18] Azarmi, R., B. Hajieghrari and A. Giglou. 2011. Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. *African Journal of Biotechnology* Vol. 10(31), pp. 5850-5855,

[19] Hatta, M., 2012. Legowo System Planting Distance Test on Growth and Results of Several Rice Varieties in Sri's Method. *Jurnal Agrista.* 16(2).

[20] Sucipto, I., A. Munif , Y. Suryadi , E.T. Tondok. 2015. Exploration of Endophytic Fungi from Rice Fields as Agents to Control Blast Disease in Rice Fields. *Jurnal Fitopatologi Indonesia.* 11(6): 211–218.

[21] IRRI 2011. Measuring Seed Germination. Postharvest Fact Sheets.

[22] IRRI. 2013. Seed Quality. Postharvest Unit, CESD. International Rice Research Institute (IRRI). quality.pdf

[23] Alison M. Smith, Samuel C. Zeeman, and Steven M. Smith Annual Review of Plant Biology 2005 56:1, 73-98

[24] Sofo, A.,G.Tataranni, C.Xiloyannis, B.Dichio and A.Scopa. Direct effects of *Trichoderma harzianum* strain.T-22 on micropropagated shoots of GiSeLa6® (Prunus cerasus × Prunus canescens) rootstock. *Environmental and Experimental Botany* 76 (2012) 33–38

[25] Hoyos-Carvajal L, Orduz S, Bissett J. Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma. Biol Control*. 2009;51:409-416.

[26] Linch, J.M.,K.L.Wilson., M.A. Ousley and J.M. Whipps. 1991. Response of lettuce to *Trichoderma* treatment .Vol.12 (2):59-61

[27] Martínez-Medina, A., Del Mar Alguacil, M., Pascual, J.A. et al. J Chem Ecol (2014) 40: 804.