Germination responses of local lowland rice variety Sirandah Kuning to application of some *Trichoderma* strain

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Abstract. Increasing rice production through intensification of the energy input in the form of chemical fertilizers and pesticides has adverse impact to the environment and health. Recognizing this, the government has launched the practice of organic farming rice intensification system, known as SRI. Besides using organic inputs, components other SRI is transplanting at a young age and the planting of one bar. The last two components found no problems to be practiced on improved varieties. Because growth is relatively fast. Instead, the application of SRI on local varieties are growing relatively slower obstacles if it is moved at a young age. In connection with this, studies have been conducted to increase the growth rate of local varieties of paddy seeds in the germination period, especially with the help of some strains of indigenous *Trichoderma.* The study was conducted from October to November 2017 in the Laboratory of Microbiology Department of Biology, Universitas Negeri Padang. A randomized block design was used in this study with 6 treatments and 4 replications. The data were processed by analysis of variance and a further test Duncant`s. The results showed that the five strains *Trichoderma* did not affect the germination percentage. Tu is the best strain because it can increase the rate of germination and seed vigor index of rice significantly, whereas *Trichoderma asperillum* (Ta) only affect the vigor index significantly.

1. Introduction

The application of green revolution to meet the domestic demand for rice from the use of improved varieties and is supported by high chemical synthetic inputs such as fertilizers and pesticides has led to a variety of adverse impact. Utilization improved varieties of high yielding intensified threat local presence. The use of synthetic pesticides has affected not only the environmental balance [1], but also harmful to the health. Every year in countries emerging there are 75,000 people suffering from poisoning insecticide and 14,000 fatalities [2]

Recognizing the impact of the use of synthetic chemical in plant cultivation is quite large, then since 2010 the government has launched a program of organic farming [3].Organic farming practices are a form of sustainable agriculture that is environmentally friendly and safe products for health because it does not use synthetic chemical fertilizers and pesticides [4]. One method of organic farming that developed at this time is the System of Rice Intensification, better known as SRI method. Implementation of SRI covers seed selection, seeding, transplanting rice with younger age, the number of seedlings planted only one, use

little water and use of organic fertilizers and biological agents as controlling pests and diseases [5]. Local varieties of rice seeds at a young age will have problems if they were transplanted to the field. Germination is a critical phase in the plants life cycle, because very sensitive to injury, disease and environmental stress [6]. Therefore it is necessary to attempt to increase the growth rate by utilizing biofertilizer. Biofertilizer is one of the environmental-friendly alternatives to increase of soil fertility and the crops productivity and yield without causing harmful environmental effects [7]. Biofertilizer is materials containing microorganisms that colonize the rhizosphere of plants and promote the availability of primary nutrients and or stimulate the growth growth [8]. Microbes that can be used as biofertilizer derived from the group of Plant Growth Promoting Rhizobacteria (PGPR) or fungi Plant Growth Promoting Fungi (PGPF). Inoculating PGPR is one potential way to reduce the impact of the application of chemical fertilizers in the long term [1]. Rhizobacteria increase growth and yield through several mechanisms including produce growth hormone auxin, gibberellins and cytokinins [9]. Applications *pseudomonads flourescen* shown to increase the growth of lowland rice and upland rice [10,11]. Plant Growth Promoting Fungi (PGPF) is a non-class soil fungal filament pathologies have beneficial effects for the plant. Fungi that includes PGPF include *Trichoderma, Fusarium, Penicillium* and *Phoma [12]*. *Trichoderma spp.* potentially be used to improve germination, vigor, growth, physiological properties and yield of rice plants [13]. Inoculation of rice plants with *T. asprellum* real SL2 increased plant height, root length, fresh weight, number of leaves and biomass compared with the controls [[14]. Increased plant growth occurs by producing phytohormones, phosphate solubilization, nitrogen fixation, degradation of cellulose and the production of siderophore [15]. Applications *harzianum* also been reported to increase the growth of seedlings, roots and shoots lenght, wet and dry weight of roots rice seedlings [16]

Although many reports indicate that *Trichoderma* can enhance plant growth, but some studies also give different results. Application *of Trichoderma sp.* no effect on the growth and yield components of tomato plants significantly [17]. Suspension application of conidia *of T. harzianum* and *T. virens* with seed soaking technique also does not affect the germination of seeds of cocoa, tomatoes and soybeans [18]. Thus concluded that the plants have different responses to *Trichoderma.* In addition, isolates *Trichoderma* also affect the response of plants. Isolates of *T. harzianum* give a different effect on taking nutrients and growth of rice seedlings [19]. Among the 10 isolates, only two best isolates the same effect to the production of dry matter, height of plant, length of root and IAA production of rice[20]. This study was conducted to evaluate the response of local rice seed germination to the strains *Trichoderma* isolated from several lowland rice rhizosphere.

2. Materials and Methods

Strains of *Trichoderma* used in this research were isolated from the different rice rhizosphere in Solok district, West Sumatra. The experiment was conducted at the Microbiology Laboratory and Plant Physiology Laboratory, Biology Department, Faculty of Mathematics and Science, Universitas Negeri Padang, from September - October 2017. The study was designed with a complete randomized design with six treatments and four replications. Before the seeds are sown into the tray seedlings, planting medium 333 garden soil mixed with 167 grams of manure and put into the seedling tray measuring 17x12x5 cm. Furthermore, the media was given water, stirring to form a slurry texture.

2.1. Suspension preparation of isolates Trichoderma

Suspension *Trichoderma* were made by pouring 10 mL in Petri dishes containing *Trichoderma* up regardless of the mycelium spores. A solution containing spores *Trichoderma* were transferred into a test tube. The solution was homogenized with a vortex so that it becomes a suspension. Subsequently, the solution was diluted to 10^{-3} and the density of the spores is calculated using a hemocytometer. The number of spores used are 10^{7} spores / ml. Spore density is calculated using the formula;

$$Conidial\ density = \frac{number\ of\ conidial\ x\ 5\ x\ dilution\ factor}{haemocytometer\ volume}$$

2.2. Preparation Seed Rice

Seeds to be used is a compact seed. Compact seed obtained by immersion in a beaker glass containing water. The seeds are floating taken out and discarded, while the seeds were submerged taken and used [21]. Seed of local lowland variety were surface sterilized by soaking with ethanol 70%, followed by 5% sodium hypochlorite and rinse by water sterilized. Fifty grains of rice seeds were selected for each treatment then soaked in the respective *Trichoderma* spp. in a flask containing 10^7 spores / mL spore suspension for 30 min. Rice seeds soaked in sterilized distilled water served as control. The treated seeds were incubated for 5 days in sterilized plastic tray at room temperature each tray was irrigated with sterilized water [13]

2.3. Giving Treatment

Fifty grains of rice seeds were sterilized soaked respectively in a test tube containing 10^7 spores / ml *Trichoderma* accordance with the treatment for 24 hours. The treatments are *T. asperillum*, *Trichoderma* strains RE, TS, SU, SB and control. On the control, seeds are soaked in tubes containing sterile distilled water. Seeds that have been treated sowing in seedling tray [13]. All units are prepared in accordance layout experiment and incubated at room temperature in the Laboratory of Plant Physiology.

2.4. Observations

2.4.1. Germination rate. Seed germination is is the average rate of the sheer number of seeds that germinate until 7 days. Germination rate evaluate by collecting fifty seeds per replicate were placed in plastic tray with soil media. Germination count was recorded everyday for 7 days after seeding (DAS). Germination was recorded on the 7th and was expressed as following [22].

$$Germination\ Rate\ (\%) = \frac{seed\ germination}{total\ seed}\ x\ 100\%$$

2.4.2. Speed of germination. Speed of germination (SG) seeds were observed every day from the first day until the seventh day after germination and is calculated by the formula:

$$SG = \frac{\text{the seeds that germinate 1st day}}{1st\ day\ of\ observation} + \cdots + \frac{\text{the seeds that germinate 7th day}}{7th\ day\ of\ observation}$$

*2.4.3. Seed Vigor Index***.** Seed vigor index of the seedlings was calculated after 7 days by multiplying the rate of germination with a length of seedling [23]

$$SVI = germination\ rate\ (\%) x\ leght\ of\ sprout\ (shoot\ lenght + root\ lenght)$$

3. Result and Discussion

3.1. Germination rate

Percentage germination ranging from 81 in Tb isolate up to 97% in Tu isolates. Referring to the requirements of a good seed, then there is no difference in the percentage of germination between control with isolates application. This caused the seed germination rate includes good if the number is 80% or more. The results of the data analysis also showed the same thing which is the percentage of germination was not influenced by the treatment of *Trichoderma,* because there is no difference in the percentage of germination between the treated *Trichoderma* and control (Figure 1). Nevertheless, the seed germination rate with treatment Ta, Te and Tu higher and significantly different from the strain Ts and Tb

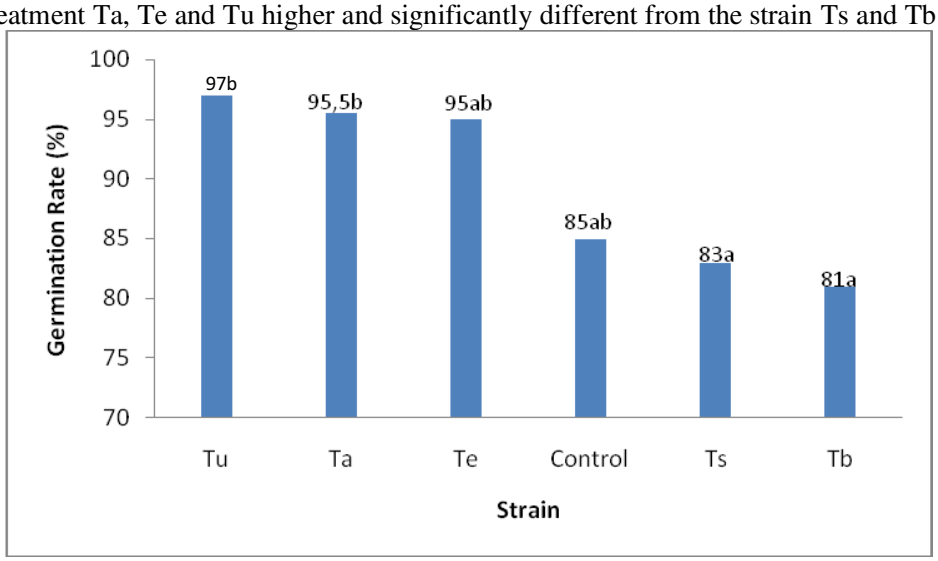

Figure 1. The rate of germination of rice seeds with application of *Trichoderma*

Isolate potential differences in increasing the growth related to the diversity of the existing properties of each isolate. Plant growth promoting potential varies among different *Trichoderma* species and strains [24]. Not all of isolates *Trichoderma* potentially increase germination. This is due to variations in properties owned diversity among the isolates or strains. Differences strains *Trichoderma* produce the same metabolite have been reported. Production of siderophore by nine strains of *Trichoderma* was studied. Under deficiency of iron the culture filtrate of all strains contained coprogen, coprogen B, and ferricrocin. Besides that, *T. pseudokoningii* and *T. longibrachiatum* produced siderophores of the fusigen type. Siderophores yields varied from 270 mg / l to 2080 mg / l. There was no siderophore could be detected in extracts of the mycelia of *T. longibrachiatum* and *T. pseudokoningii* [25]. Applications *Trichoderma* not always responded with an increase in germination of the plants. Seed germination rate was not affected by *Trichoderma* application [19]. Application of *T. virens* or *T. harzianum* only able to grow 30% cacao seed germination. The number is the same as that obtained in the control germination. Even application *Trichoderma* on soybean seed tomatoes and it resulted in a lower germination percentage compared with the control [18]. Application of *Trichoderma* sp. no l effect on the growth and yield components of tomato plants significantly [17]. Suspension application of conidia *of T. harzianum* and *T. virens* with seed soaking technique also does not affect the germination of seeds of cocoa, tomatoes and soybeans [18]. Five isolates inoculation of *Trichoderma* on wheat seed germination percentage not substantially different from the control [[7].

3.2. Germination speed

Percentge of Germination usually used as an indicator viability of seed. Thus, this value is not sensitive enough to detect the slow reduction in seed in field conditions. On the other hand, germination speed is better reflect changes in seed vigor [26]. The results showed that the speed of germination of rice seeds is influenced by the type or strain of *Trichoderma.* Of the five *Trichoderma* tested, only Tu isolate highest velocity and significantly different compared with four other isolates and controls. In contrast, four other isolates showed the speed of germination is not different compared with the control (Figure 2).

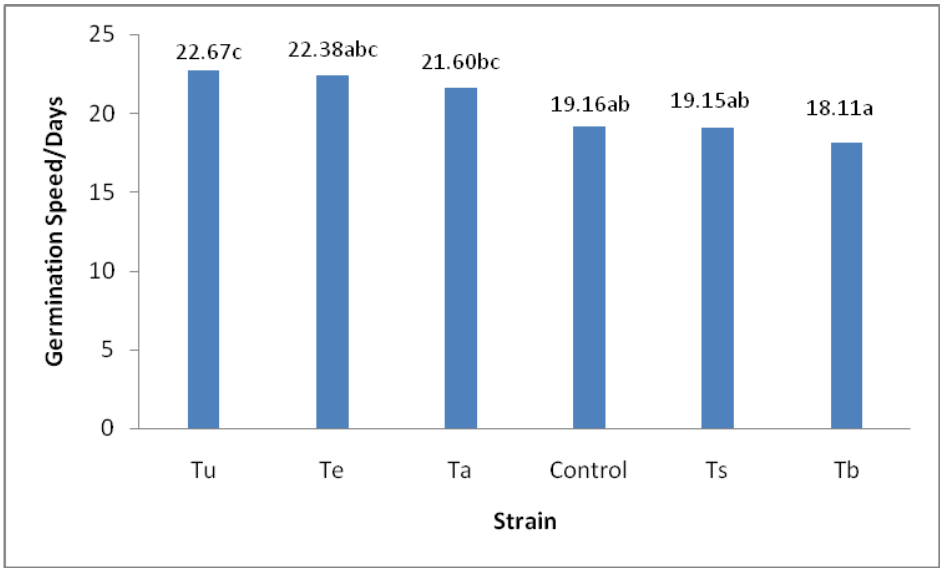

Figure 2. Speed rice seed germination with application *of Trichoderma*

Effect of *Trichoderma* in enhancing the growth varies among species or strains. *T. harzianum* T22 strain treatment tomatoes increased the speed of seed germination [26]. *T. virens* is able to produce plant growth regulator (PGR) in the form of IAA (Indole Asetic Acid) that could increase plant growth by promote root growth rate [27]. Plants respond differently to the application *of Trichoderma.* Any metabolites production was a characteristic of specific strains, because the ability metabolites production varied within species greatly. Seven *Trichoderma* isolates promote the growth of bean seedlings significantly. However, metabolite production in these isolate widely varied in strains.Ssome isolates did not produce any of the assessed growth-promoting metabolites [28]. *Trichoderma* strains G124-1, G46-3 and G46-7 had negative effects on the highest branch decreased length in *Silybum marianum* which decreased 63.7, 46.5, 40.33 and 30.5, respectively [29]

3.3. Seed Vigor Index

Lowest seed vigor index found in Tb treatments and the highest in the Tu. Of the five *Trichoderma* treatment is given, the strain Te, Ts and Tb showed lower vigor and no different than control. In contrast, Ta and Tu give vigor higher seed and different from the control (Figure 3). Thus it can be known that *Trichoderma* strain unequal effect on seed vigor.

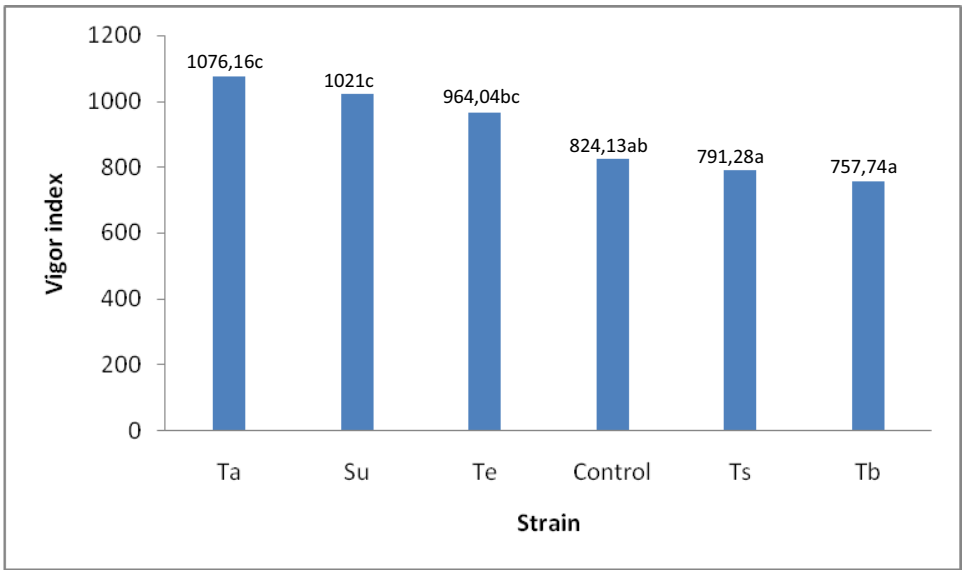

Figure 3. The rice seed vigor index with application *of Trichoderma*

Role of *Trichoderma* in enhancing the seed vigor has been reported by few researchers. *Trichoderma* were able improve health and vigor of plants [30]. *T. harzianum* strain T22 increased vigor index of tomatoes [26]. Germination and vigor index of rice seed treated with *Trichoderma* sp. higher than the non-treated rice seed [13]. *Trichoderma* including one group phytostimulant with some way mechanism. The ability of *Trichoderma* spp. produce phytohormone is an important factor in increasing the growth of rice [31]. Mechanism of phytostimulation by *Trichoderma* involves multilevel communication with root and shoot systems, as it releases into the rhizosphere auxins, small peptides, volatiles and other active metabolites, the which promote root branching and nutrient uptake capacity [32]. IAA is the most widely naturally occurring auxin in vascular plants, and it has great importance during lateral and adventious roots initiation and emergence, and shoot development. Levels of IAA in both leaves and roots increased significantly (148 and 122% of the un-inoculated plants, respectively) after the inoculation with *Trichoderma* T22 [33]. Increased vigor can also occur because of its role in the supply of plant nutrients. *Trichoderma* affect plant growth due to its ability in nutrient solubilization [34], phosphate solubilization [35], the production of siderophore [25] and celloluse degrading [36]. *Trichoderma* application use of nitrogen fertilizers were able to save up to 40-50% with no reduction in yield [37]. Certain strains of *Trichoderma* is effective as biostimulant [38]. T.*harzianum* can improve seed germination, root and shoot length and root dry weight of wet rice seeds [16].

4. Conclusion

Based on the research that's done concluded that the strain or species of *Trichoderma* give a different effect on the parameters. Five treatments are not able to increase the percentage of germination. Conversely, *Trichoderma* strain Tu can promote the rate of germination and vigor index, while *T. asperillum* (Ta) only able to increase the vigor and give the same results with the strain Tu.

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