Application of local microbes increases growth and yield of several local upland rice cultivars of Southeast Sulawesi, Indonesia

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Abstract. Rice is the major food commodity in Indonesia and many other countries, as the main source of carbohydrate. Rice production must be increased continuously to meet food needs, one of which is by utilizing largely available dry land areas. Two important factors required to increase rice production on marginal soils are the use of high-production adaptive varieties and biological agents. It is necessary to conduct research on the application of local microbes to the cultivation of upland rice cultivars, with the aim to determine the best genotypes and/or suitable microbes. This research was carried out at the Research Field and laboratories of Faculty of Agriculture, University of Halu Oleo, from April 2020 to April 2021. The first tested factor was biological agents (local microbes), consisting of three levels, namely: without microbes (M0), the fungus Trichoderma sp. (M1), and the bacteria Pseudomonas sp. (M2). The second factor was the upland rice cultivar, which consisted of five levels, namely: V1 (Tinangge cultivar), V2 (Enggalaru cultivar), V3 (Bakala cultivar), V4 (Momea cultivar), and 1 nasional variety, namely V5 (Inpago-12 variety), as check variety. The research results showed that the interaction between local microbes Trichoderma sp. (M1) and Pseudomonas sp. (M2) on Tinangge (V1) and Momea (V4) cultivars gave a better effect on crop production. The best cultivar based on the growth and yield variables was generally obtained from the Momea cultivar (V4), although in many variables it was not significantly different from the Tinangge cultivar (V1). These cultivars could be further studied and possibly developed for promising cultivars in Kendari areas.

1. Introduction
Rice is the main food commodity for people in Indonesia and in many Asian countries. Rice as a staple food is the main source of carbohydrate intake [1]. The Central Bureau of Statistics [2] reported that rice production in 2019 was 519.71 thousand tons of dry milled unhulled rice. This production decreased by 19.17 thousand tons (3.56%) compared to the production in 2018. The National Central Bureau of Statistics [3] also reported a decline in national rice production in Indonesia, by 2.63 million tons (7.75%) in 2019 compared to 2018’s production.
The decline in rice production was partly due to the narrowing of productive agricultural land as a result of the conversion of agricultural land, and the lack of the use of high-yielding varieties [4, 5]. Efforts to increase rice production must continue to meet food needs, one of which is the use of dry land. The prospects for the development of dryland rice (with upland rice cultivation) are still very large, with an area of dry land that is still available [6]. Southeast Sulawesi itself has an area of dry land reaching 2.3 million hectares [7].

In addition to the availability of extensive dry land, Southeast Sulawesi also has an abundant source of upland rice diversity. Local rice is still an asset of genetic resources in providing adaptive superior varieties [5]. Characterization and evaluation of germplasm is essential in the breeding program [8]. Through characterization activities, the superior traits of the germplasm can be identified so that genotypes of potential genotypes can be obtained [9, 10]. Germplasm characterization is also important in the conservation and preservation of local varieties [11].

Diversity in various plants is the result of the interaction between genetic, environmental and plant development factors. Superior varieties and cultivation techniques are the main components in increasing rice production. According to [12], upland rice varieties have superior characteristics such as high yields, resistance to major diseases, and early maturity, so they are suitable to be developed in certain cropping patterns and have a good taste (fluffy) with relatively high protein content.

The cultivation techniques in an effort to increase upland rice production include the provision of organic matter, the selection of high-production adaptive varieties and the use of biological agents on marginal soils. Various microbial species (bacteria and fungi) have been isolated and evaluated for their effectiveness as active ingredients in biological fertilizers and pesticides [13]. *Pseudomonas* sp. and *Trichoderma* sp. are antagonistic microbes that can help fertilize the soil and are widely used as biological control agents, or are widely used to control plant pathogens, involved in nitrogen fixation, inorganic phosphate solvents, iron complexation, and phytohormone synthesis [14].

One of the functional microorganisms widely known as soil biological fertilizer is the fungus *Trichoderma* sp. [15]. This fungus, apart from being a decomposer organism, can also be a biological agent and plant stimulator. Other microbes that can be used to increase the growth and production of rice plants is *Pseudomonas* sp. This type of microbe is also an antagonist, because it is able to produce antibiotic compounds (antifungal), siderophores and other secondary metabolites that protect plants and inhibit fungal activity [16].

Based on the description above, it is necessary to conduct research related to the application of local microbes to the cultivation of local upland rice cultivars, with the aim of knowing the interaction between local microbes and genotypes on the performances of several local upland rice cultivars, and to determine the best genotype that can be developed in Kendari City, Southeast Sulawesi.

2. Materials and methods

2.1. Research implementation and materials

This research was carried out at the Field Laboratory II, Agronomy Laboratory, and Phytopathology Laboratory, Faculty of Agriculture, Halu Oleo University, from April 2020 to April 2021.

The materials used were 4 (four) upland rice cultivars from Southeast Sulawesi, namely: V1 (Tinangge cultivar), V2 (Enggalaru cultivar), V3 (Bakala cultivar), V4 (Momea cultivar) and one national variety, namely V5 (Inpago-12 variety), for the control variety.

2.2. Land preparation

Land preparation began with clearing the grasses, plowing, making drainage and plots, with a size of 1 m x 1.25 m. Basic fertilization was done by giving 1.25 kg of organic cow dung per plot and 0.25 kg of lime (dolomite) per plot, 2 weeks before planting.
2.3. Microbe preparation
The local microbe used was the endophytic fungus *Trichoderma* sp. (M1) (Phytopathology Laboratory’s collection) purified on Potato Dextrose Agar (PDA) media. After 7 days of incubation, the spores of *Trichoderma* sp. isolates were harvested by adding 10 mL of distilled water to the petri dish filled with *Trichoderma* sp., then the surface of the isolate was ground using a grinding rod. The local microbe used for M2 treatment was *Pseudomonas* sp. bacteria (collection of Agronomy Laboratory). The isolates were grown on TSA media and then incubated for 48 hours. The growing bacterial colonies were suspended in 200 mL of sterile distilled water.

2.4. Microbe application
The first application was carried out on sterilized seeds (using 70% alcohol, 2% NaOC; and sterile distilled water 3 times), by soaking the seeds in the microbial solution of *Trichoderma* sp. or *Pseudomonas* sp. for 1-2 hours. The second application was done by mixing *Trichoderma* sp. with 3 kg of cow dung fertilizer, then apply to plants as much as 5 grams per planting hole. Meanwhile, for the treatment of *Pseudomonas* sp., it was applied by spraying the microbial solution directly to all parts of the plant, as much as 265 mL per plot, when the plant was 14 days old.

2.5. Planting and fertilization
Planting was done by making planting holes as deep as 1-2 cm and then sowing 5-6 seeds of rice seeds per planting hole. The spacing used was 20 cm x 25 cm; so that there were 25 planting holes per experimental plot. Fertilization was done by dipping around the plant. The fertilizers used were urea and NPK fertilizer with a recommended dose of 100 kg urea per ha, or 0.012 kg per plot, NPK as much as 250 kg per ha, or 0.031 kg per plot. Fertilization was carried out four times during the growth period.

2.6. Grain harvesting
Harvesting was done when at least 85% of the grain in each clump had turned yellow. Harvesting was done by cutting the upland rice panicles using a harvesting tool (sickle).

2.7. Observed variables
The observed growth and yield variables were plant height (cm), number of leaves per plant (strands), leaf area (cm²), according to [17], number of tillers (tiller) per plant, age 50% of flowering plants (days), harvest age (days), panicle length (cm), number of productive tillers (tiller), flag leaf area (cm²), total grain per panicle (grain), percentage of grain content per panicle (%), weight of 1000 grains (g), weight of grain per clump (g), grain color, grain shape and grain size (based on the IRRI class system [18]).

2.8. Research design and data analysis
This study used a factorial design in a randomized block design (RBD), consisted of 2 factors. The first factor was biological agents (local microbes), consisting of three levels, namely: without microbes (M0), the fungus *Trichoderma* sp. (M1), and the bacteria *Pseudomonas* sp. (M2). The second factor was the upland rice cultivar, which consisted of five levels, namely: V1 (Tinangge cultivar), V2 (Enggalaru cultivar), V3 (Bakala cultivar), V4 (Momea cultivar), and 1 nasional variety, namely V5 (Inpago-12 variety), as check variety. Each group consisted of 15 treatment combinations, which were repeated 3 times, resulting in a 45-unit experiment, as in [19]. Research data were analyzed by means of variance, and continued with Duncan’s multiple range test (DMRT), with 95% confidence level (α).

3. Results and discussion

3.1. Research results
The research results on some growth and yield parameters of the five upland rice genotypes tested are presented in table 1 to table 6, along with the characteristics of the rice grains (table 7 and 8).
growth and yield performances of the five genotypes were varied. In most cases, two upland rice genotypes namely Tinangge (V1) and Momea (V4) generally showed better growth and yield performances as compared to the other genotypes. On the other hand, microbe treatments M1 (Trichoderma sp.) and M2 (Pseudomonas sp.) significantly improved the upland rice performances.

**Tabel 1.** Averages of plant height (cm), number of leaves (strands), total leaf width (cm²), and number of tillers (plant) at the age of 84 days after sowing (DAS), of several genotypes of local upland rice from Southeast Sulawesi treated with different local microbes.

| Microbe treatments | Plant height (cm) | Leaf number per plant (strands) | Total leaf width per plant (cm²) | Tiller number (plant) |
|--------------------|-------------------|-------------------------------|---------------------------------|----------------------|
| M0                 | 132.5 b           | 35.3                          | 3320.0                          | 9.8 b                |
| M1                 | 135.5 a           | 37.1                          | 3453.8                          | 10.5 ab              |
| M2                 | 135.0 a           | 37.0                          | 3551.2                          | 10.7 a               |

*Note: The numbers followed by different letters in the same column are significantly different based on the DMRT with a 95% confidence level*

**Tabel 2.** Averages of flowering age (days), productive tiller number (plant), number of grains per panicle, and percentage of filled grain per panicle of several genotypes of local upland rice from Southeast Sulawesi treated with different local microbes.

| Microbe treatments | Flowering age (days) | Productive tiller number (plant) | Number of grains per panicle (grain) | Percentage of filled grain per panicle (%) |
|--------------------|----------------------|----------------------------------|-------------------------------------|-------------------------------------------|
| M0                 | 98.5 a               | 7.4 b                            | 176.8 b                             | 71.8 b                                    |
| M1                 | 96.6 b               | 7.8 ab                           | 186.1 a                             | 79.8 a                                    |
| M2                 | 96.0 b               | 8.4 a                            | 184.0 ab                            | 79.1 a                                    |

*Note: The numbers followed by different letters in the same column are significantly different based on the DMRT with a 95% confidence level*

**Tabel 3.** Averages of dry grain weight per clump (g) and one-thousand-grain weight of several genotypes of local upland rice from Southeast Sulawesi treated with different local microbes.

| Microbe treatments | Dry grain weight per clump (g) | 1000-grain weight (g) |
|--------------------|-------------------------------|-----------------------|
| M0                 | 25.8 b                        | 27.4 b                |
| M1                 | 29.1 a                        | 28.8 a                |
| M2                 | 27.6 ab                       | 27.9 ab               |

*Note: The numbers followed by different letters in the same column are significantly different based on the DMRT with a 95% confidence level*

**Tabel 4.** Averages of plant height (cm), number of leaves (strands), total leaf width (cm²), and number of tillers (plant) at the age of 84 days after sowing (DAS), of several genotypes of local upland rice from Southeast Sulawesi.

| Genotype treatments | Plant height (cm) | Leaf number per plant (strands) | Total leaf width per plant (cm²) | Tiller number (plant) |
|---------------------|-------------------|---------------------------------|---------------------------------|----------------------|
| V1                  | 102.4 b           | 26.2 c                          | 2647.2 c                        | 8.0 b                |
| V2                  | 100.4 b           | 29.5 b                          | 3007.4 b                        | 8.2 b                |
| V3                  | 106.8 a           | 24.3 c                          | 2413.5 c                        | 6.3 c                |
| V4                  | 102.7 b           | 35.1 a                          | 3652.9 a                        | 10.4 a               |
| V5                  | 91.4 c            | 21.6 d                          | 1185.3 d                        | 5.8 c                |

*Note: The numbers followed by different letters in the same column are significantly different based on the DMRT with a 95% confidence level*
**Table 5.** Averages of flowering age (days), productive tiller number (plant), number of grains per panicle, and percentage of filled grain per panicle of several genotypes of local upland rice from Southeast Sulawesi.

| Genotype | Flowering age (days) | Productive tiller number (plant) | Number of grains per panicle (grain) | Percentage of filled grain per panicle (%) |
|----------|----------------------|----------------------------------|--------------------------------------|------------------------------------------|
| V1       | 76.3 b               | 5.4 b                            | 158.8 a                              | 60.9 a                                   |
| V2       | 74.3 c               | 5.7 b                            | 111.9 d                              | 59.8 ab                                  |
| V3       | 76.8 b               | 5.8 b                            | 128.2 c                              | 55.2 b                                   |
| V4       | 80.1 a               | 8.1 a                            | 143.6 b                              | 53.2 b                                   |
| V5       | 56.3 d               | 4.6 c                            | 141.1 b                              | 59.4 ab                                  |

Note: The numbers followed by different letters in the same column are significantly different based on the DMRT with a 95% confidence level.

**Table 6.** Averages of dry grain weight per clump (g) and one-thousand-grain weight of several genotypes of local upland rice from Southeast Sulawesi treated with different local microbes.

| Genotype | Dry grain weight per clump (g) | Flag-leaf width (cm²) | Panicle length (cm) | Harvest age (days) | 1000-grain weight (g) |
|----------|--------------------------------|-----------------------|---------------------|--------------------|-----------------------|
| V1       | 21.5 b                         | 39.0 ab               | 19.2 b              | 94.8 c             | 21.44 ab              |
| V2       | 20.2 b                         | 35.9 ab               | 20.5 ab             | 96.0 c             | 21.6 a                |
| V3       | 16.3 c                         | 37.1 ab               | 20.3 ab             | 100.1 b            | 20.8 b                |
| V4       | 26.1 a                         | 41.1 a                | 21.5 a              | 102.8 a            | 21.3 ab               |
| V5       | 19.1 b                         | 32.7 b                | 16.8 c              | 77.7 d             | 20.0 b                |

Note: The numbers followed by different letters in the same column are significantly different based on the DMRT with a 95% confidence level.

**Table 7.** Characteristics of grain colour of up-land rice genotypes.

| Genotypes          | Score | Criteria                          |
|--------------------|-------|-----------------------------------|
| Tinanggea (V1)     | 3     | Brown stripes on a straw yellow background |
| Enggalaru (V2)     | 1     | Golden yellow                     |
| Bakala (V3)        | 3     | Brown stripes on a straw yellow background |
| Momea (V4)         | 0     | Straw yellow                      |
| Inpago-12 (V5)     | 1     | Golden yellow and golden stripes on a straw yellow background |

*) Based on IRRI (2013)

**Table 8.** Classification of grain shape and size of up-land rice genotipes.

| Genotypes          | Criteria |
|--------------------|----------|
|                    | Shape    | Size     |
| Tinanggea (V1)     | Oval     | Medium   |
| Enggalaru (V2)     | Oval     | Medium   |
| Bakala (V3)        | Oval     | Medium   |
| Momea (V4)         | Oval     | Long     |
| Inpago-12 (V5)     | Round    | Bigger   |

*) Based on IRRI (2013)
3.2. Discussion

The research results showed that the treatment of local microbes and different cultivars resulted in different growth and production of local upland rice plants, as in line with the results of previous studies [20]. The interaction of microbial and cultivar treatments in general had no significant effect on the observed variables of growth and production of local upland rice, except for the number of leaves at 28 days of age, leaf area of 14 and 28 days, and the number of grain contents per panicle.

The results (Table 4) showed that the highest average plant height was generally obtained in the Bakala cultivar (V3). Differences in plant height are thought to be influenced by plant genetic factors, as [21] reported that differences in plant height were caused by differences in genetic characteristics and traits.

In addition to having different plant heights, different cultivars also showed differences in the number of tillers (Table 4). The highest mean of tillers was generally obtained in the cultivar Momea (V4). The difference in the number of tillers occurred due to the ability of each cultivar to produce tillers [22]. One of the things that greatly affects this is the number of nodes or stem segments of each cultivar. The number of tillers in the Momea cultivar (V4) will have implications for increasing the number of leaves of upland rice plants, as produced in this study. In general, the highest number of leaves was found in the cultivar Momea (V4) (Table 4). This is because each tiller will form leaves.

Measurement of leaf area of several local upland rice cultivars showed that there was variation in the observed leaf area morphology (Table 4). The highest mean of leaf area was obtained in the cultivar Momea (V4). According to [23], the increase in plant leaf area is caused by the increasing number of tillers and the increasing area of each leaf itself, which is also controlled by the environment.

Cultivars that have a shorter 50% flowering age, the maturity age of the variety is also relatively shorter [24], or commonly referred to as early maturing [25]. The sooner rice plants reach 50% flowering age, then the harvest age will be faster. Based on the results of the research, flowering age (Table 5) and harvesting age (Table 6) were highest in the Momea cultivar (V4), while the national variety Inpago-12 (V5) had the lowest value. Plants will show ripe harvest if the total energy adopted has reached a certain limit, which is generally caused by genetic factors [26].

The Momea cultivar (V4) had the longest average panicle length of 21.52 cm (Table 6). The increase in panicle length was influenced by genetic factors [27] and the adaptability of the cultivar to the growing environment. The panicle length component is the main supporting factor for yield potential, the longer the panicle the more opportunities to produce grain [28]. The length of the panicle is the seat of the grain whose presence is very important. If the panicle is damaged, then the tillers will not produce rice grains.

The results showed differences between cultivars in terms of the number of productive tillers and the number of grains per panicle (Table 5). The highest number of productive tillers was obtained in the Momea cultivar (V4) with an average of 8.05 tillers, and the lowest was obtained in the Inpago-12 variety. According to [29], the number of tillers will be maximized if the plant has good genetic characteristics. Genotypes that have many tillers tend to have high clump production. The large number of tillers is one of the desirable traits in the selection and assembly of new varieties. The results (Table 5) showed that the highest average number of grains per panicle and the number of filled grains per panicle (158.78 grains) was obtained in the Tinanggea cultivar (V1), and the lowest (111.91 grains) was obtained in the Enggalaru cultivar (V2). The difference in the number of grains per panicle and the number of filled grains per panicle produced is caused by genetic factors of each cultivar [30]. In addition, environmental factors also play a role in increasing the rate of photosynthesis to produce food (photosynthetic). Photosynthesize produced will be stored in the stem and leaf tissues, which is then translocated to the grains. The results (Table 5) showed the highest average value of the percentage of filled grain (60.94%) was obtained in the Tinanggea cultivar (V1). The highest average weight of 1000 grains (21.6 g) was obtained in the Enggalaru cultivar (V2) (Table 6), although it was not significantly different from the Tinangge (21.4 g) and Momea (21.3 g) cultivars.

The yield potential of a rice genotype is determined by the components of the grain characters, namely the number of grains per panicle and the percentage of grain content per panicle. Like panicle
length, full or pithy grain and empty grain also affected yield [31, 32]. Grain quality is also one of the selection parameters. The high quality of rice plants is reflected in the large number of filled grains and the low number of empty grains [33]. The amount of grain content is one of the important yield components used as a selection component to determine the ability of plants to produce [34]. The genetic potential of the character of the number of filled grain per panicle produced will be better if it is supported by suitable environmental conditions in the development of a plant line, namely climatic conditions with sufficient lighting for photosynthesis, adequate nutrients, and sufficient water during seed filling.

The cause of the weight of 1000 grains of grain that differed between cultivars and varieties, was influenced by conditions after flowering, such as the availability of nutrients, weather and the number of leaves. This is in line with the statement of [35] and [36] that these conditions will affect the amount of carbohydrates produced by the photosynthesis process and will then determine the size of the grain. The amount of nutrient absorption resulted in the complete filling of the grain and resulted in a higher 1000 grain weight. The yield component is a quantitative trait that affects the yield, so the high and low yields are highly dependent on the yield component components that compose it, including grain content, weight of 1000 grains and weight of harvested grain [37].

The results of the study (Table 1 and Table 2) showed that the local microbial treatment of *Trichoderma* sp. (M1) gave the highest plant response in the form of plant height at the age of 14, 28, 56 and 84 days, leaf area, number of leaves, flowering age, number of grains per panicle, percentage of grain content, and weight of 1000 grains (Table 3), although each was not significantly different than the treatment of *Pseudomonas* sp. (M2). On the other hand, local microbial treatment of *Pseudomonas* sp. (M2) gave the highest response to the number of tillers and the number of productive tillers, although it was not significantly different from the microbial treatment of *Trichoderma* sp. (M1). In addition to the genetic characteristics of cultivars, the increase in plant growth is also thought to be influenced by the direct supply of nutrients from the application of local microbes. Local microbes can stimulate plant growth with several advantages, namely fixing nitrogen, dissolving phosphate and synthesizing IAA hormones. The use of PGPR was able to increase root and stem length, dry weight of roots and stems, root development, canopy cover, N content in roots and stems of rice plants [38, 39]. The use of microbes can increase the growth and yield of several upland rice cultivars [40]. Not surprisingly, in this study there was an increase in growth and yield of several upland rice cultivars that were applied with local microbes.

4. Conclusions
Based on the research results and discussion, it can be concluded that the interaction between local microbes *Trichoderma* sp. (M1) and *Pseudomonas* sp. (M2) on Tinangge (V1) and Momea (V4) cultivars gave a good effect on crop production. The best cultivar based on the growth and yield variables was generally obtained from the Momea cultivar (V4), although in many variables it was not significantly different from the Tinangge cultivar (V1).

Acknowledgment
The authors gratefully acknowledge the financial support from Directorate General of Higher Education, Ministry of Education and Culture, Indonesia, for funding supports of the research project through the grant for Excellent Higher Education Applied Research Program 2020 dan 2021.

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