The application of biopriming using trichoderma and Streptomyces spp. on the germination stage of soybean

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Abstract. Biopriming is one way to overcome the stunted plant growth due to a decline in the quality of seed physiology by using a combination of biological agents. This study aims to improve the quality of seed germination and vigor with a combination of biological agents in the form of Trichoderma harzianum and Streptomyces spp. This research was conducted at the Laboratory of Bio-Science and Plant Reproductive Biotechnology, Department of Agriculture Cultivation, Faculty of Agriculture, Hasanuddin University, which took place from January to April 2019. This study used a Split Plot Design, the main plot was the duration of biopriming consisting of four levels, 3 hours, 6 hours, 12 hours and 24 hours. Type of microbes as subplots consisting of three levels, namely water, Trichoderma harzianum and Streptomyces spp. The results show that the duration of biopriming 12 hours with Streptomyces spp. able to produce the highest average percentage of normal sprouts (96.48%). The duration of biopriming treatment for 12 hours with water produced the highest average percentage of abnormal sprouts (15.09%). Treatment duration of biopriming for 6 hours with Streptomyces spp. able to produce the highest average length of root sprouts (11.91 cm). Streptomyces spp. gave the highest yield at an average germination rate (90.42%).

1. Introduction
Soybean (Glycine max L.) is one of the main food commodities after rice and corn. Soybeans are included in legumes which have very high levels of nutrition, especially vegetable protein. One of the causes of decreasing soybean production results is a decline in the quality of seed physiology. Irwan [1] explains the use of seeds that have poor physiological quality can reduce the number of population per unit area because soybean cultivation involves planting seeds directly in the field.

One way to overcome stunted plant growth due to a decrease in physiological quality of seeds, causing a decrease in vigor, viability, germination of seeds that have undergone deterioration that is biopriming by using a combination of biological agents. Biopriming is a seed priming technique that uses biological agents in the treatment solution [2]. According to Sutariati et al. [3], in addition to providing improvements, biopriming treatment also acts as a media for rhizobacterial inoculation of seeds. Seed invigoration through the process of biopriming with rhizobacteria provides benefits to plants, and is proven effective in increasing seed viability and vigor.

Giving biological agents that are able to improve the quality of seed germination can be call as a biopriming treatment. Nitrogen-fixing microbes that are able to be biological control agents, and microbes that are able to produce hormones. Those treatment is known as biopriming. The
combination of microbes that can be used as biopriming agents is *Trichoderma harzianum* and *Streptomyces* spp.

*Trichoderma harzianum* is able to produce induction hormones that plants need to grow. According to Contreras-Cornejo [4] states *Trichoderma* sp. is a microbe that is able to produce auxin hormone in the form of IAA which plays an important role in the process of root elongation which causes higher absorption of nutrients. So that crop production yields are also higher. *Trichoderma harzianum* is able to produce IAA (Indol-3-Acetic Acid) which plays a role in controlling physiological processes, including enlargement and cell division, stimulating seeds to germinate tissue differentiation and control vegetative growth processes.

Magda's research results [6] showed that *Streptomyces* spp. in the soil can increase root length, shoot height, root dry weight, and able to fix nitrogen, and produce IAA hormones needed by plants for the growth process. Research conducted by Tefa [7] shows that *Actinomycetes* sp. has an IAA hormone content of 89.5 ppm and gibberellin 92.5 ppm.

2. Methodology
This research was conducted at the Laboratory of Bio-Science and Plant Reproductive Biotechnology, Department of Agriculture Cultivation, Faculty of Agriculture, Hasanuddin University. This research was conducted in January to April 2019.

The tools used are 1000 mL erlemeyer flasks, 1000 mL measuring cups, petridist plates, test tubes, Laminar Air Flow, autoclaves, (Conten Chlorofil Meter-200 plus) CCM-200 plus, aerators, analytical scales, and digital cameras. The ingredients used are grobogan soybean varieties, *Streptomyces* spp., *Trichoderma harzianum*, water (aquades) 1000 mL, unraping, aluminum foil, Nutrient Broth (NB) 8 g, Bacto agar 18 g, label paper, soil, manure, paper filter, size 30-40 polybags and plastic picks. The seeds used are seeds that have been stored for 1 year. Initial quality analysis shows that the germination rate of seeds is 75%.

The method used is the Split Plot Design consisting of the main plot and sub-plot, repeated 3 times. The main plot is the duration of biopriming (P) consisting of 4 levels, namely duration of 3 hours (P1), duration of 6 hours (P2), duration of 12 hours (P3), and duration of 24 hours (P4). Subplot is a type of microbe (M) consisting of 3 types namely Water (M0), *Trichoderma harzianum* (M1) and *Streptomyces* spp. (M2).

The experiment was carried out by soaking the seeds using *Trichoderma harzianum* 1x10^7 cfu / mL, *Streptomyces* spp. 1x10^7 cfu / mL and Water for 3, 6, 12, and 24 hours according to each treatment. Then the seeds are air-dried to restore the initial weight of the seeds. After the initial weight of the seeds is returned, the seeds are germinated using a tissue. Observations included percentage of sprouts, percentage of normal sprouts, percentage of abnormal sprouts, simultaneity of growth, and length of root sprouts.

Analysis of the data used in this study is a Duncan Multiple Range Test (DMRT) at the 5% level if there is significant effect of the treatments.

3. Results

3.1. Effect of interaction between biopriming duration with different types of microbes
The results showed that there was a significant interaction between the duration of immersion with various types of microbes to normal sprouts, the number of productive branches, the number of planting pods and there was a very significant interaction which was obtained from the observation of the percentage of abnormal sprouts and root length of sprouts (table 1).

Based on the results of immersion for 12 hours using microbes *Streptomyces* spp. the highest percentage of normal sprouts in soybean plants was 96.48%. This is caused by *Streptomyces* spp. can produce IAA hormones that can affect plant growth and root differentiation. This is supported by Aryantha et al. [8] which states that *Streptomyces* spp. able to produce IAA which plays a role in stimulating plant growth.
Table 1. Average percentage of normal sprouts (%) in the treatment of Biopriming duration and types of microbes (Trichoderma harzianum and Streptomyces spp.) in soybean plants (Glycine max L.)

| Sprouts | Duration Biopriming (P) | Microbe Microbes (M) | Duncan0.05 |
|---------|-------------------------|----------------------|------------|
|         | Control (M0)            | Trichoderma harzianum (M1) | Streptomyces spp. (M2) |         |
| Normal sprouts | 3 hours (P1) | 89.91(3.18)b^x | 92.97(3.15)c^z | 91.20(3.12)b^y | 2.29 |
|         | 6 hours (P2) | 93.24(3.19)c^x | 86.67(3.15)c^z | 91.67(3.12)b^y | 2.40 |
|         | 12 hours (P3) | 84.91(3.18)c^x | 91.67(3.12)b^y | 96.48(3.15)c^x | 2.47 |
|         | 24 hours (P4) | 91.29(3.18)c^y | 90.00(3.15)c^z | 94.91(3.12)a^x | 2.47 |
| Abnormal sprouts | 3 hours (P1) | 10.09(3.18)a^x | 7.02(3.15)c^z | 8.79(3.12)b^y | 0.36(0.066) |
|         | 6 hours (P2) | 6.75(3.18)c^x | 10.00(3.15)a^x | 8.33(3.12)b^y | 0.38(0.070) |
|         | 12 hours (P3) | 15.09(3.18)a^x | 8.33(3.15)c^z | 3.52(1.88)c^z | 0.39(0.072) |
|         | 24 hours (P4) | 8.70(3.18)c^x | 8.33(3.15)c^z | 5.09(2.27)c^z | 0.40(0.070) |
| Duncan0.05 | 0.24(0.046) | 0.26(0.049) |         |         |

Note: The numbers followed by letters are not the same in columns (a, b, c) and rows (x, y, z) mean significantly different at Duncan's advanced test level α = 0.05 (Numbers in the Transformation Data Brackets √ (X +0.05)).

The highest percentage of abnormal sprouts obtained in the immersion for 12 hours without microbes was 3.89%. This is because the soybean seeds used are of a quality that has decreased physiologically and there is no specific treatment of quality deterioration, such as the addition of microbes that can increase the germination and seed vigor such as Trichoderma harzianum and Streptomycetes spp. which is able to produce IAA and Gibberellins, resulting in high abnormal numbers of sprouts. This is consistent with the opinion of Herlina and Aziz [9] which states that the process of seed germination is a process that determines plant growth. Where the germination of plant seeds is influenced by several factors, including the type of seed and the quality of the seeds used, the initial treatment of seeds and the gibberellin hormone content that is in the seed.

Soaking seeds for 12 hours is thought to be the most effective time for seeds to be able to improve and complete the process of metabolism. Germination is an illustration of the ability of seeds to develop into normal plants [10]. The duration of soaking is related to the process of imbibition in the seed. Because during soaking there is an improvement and completion of the process of seed metabolism [11].

Table 2. Average length of sprout roots (cm) in the treatment of Biopriming duration and types of microbes (Trichoderma harzianum and Streptomyces spp.) in soybean plants (Glycine max L.)

| Biopriming Duration (P) | Type of Microbes (M) | Duncan0.05 |
|-------------------------|----------------------|------------|
|                         | Control (M0)         | Trichoderma harzianum (M1) | Streptomyces spp. (M2) |         |
| 3 hours (P1)            | 11.65(1.10)b^x | 8.75(0.99)c^z | 10.91(1.08)b^y | 0.40(0.015) |
| 6 hours (P2)            | 9.52(1.02)b^x | 10.87(1.07)c^y | 11.91(1.11)c^z | 0.41(0.016) |
| 12 hours (P3)           | 8.11(0.96)c^z | 10.53(1.06)c^z | 9.29(1.01)c^z | 0.43(0.016) |
| 24 hours (P4)           | 9.09(1.00)c^x | 3.79(0.68)c^z | 9.58(1.02)c^x |         |
| Duncan0.05              | 0.25(0.010) | 0.26(0.011) |         |         |
Note: The numbers followed by letters are not the same in columns (a, b, c) and rows (x, y, z) mean significantly different at Duncan's advanced test level α = 0.05 (Numbers in data brackets Transformation log X + 1).

Soaking for 6 hours using microbes Streptomyces spp. able to produce the highest average root length compared to other treatment combinations that is 11.91 cm. This is caused by Streptomyces spp. able to fix nitrogen in the soil and produce auxin hormone in the form of IAA needed for the process of plant growth. This is consistent with the results of Magda’s [6] study stating that the inoculation of Streptomyces spp. in soil media can increase root length, shoot height, root dry weight and can fix nitrogen, producing auxin that plants need for growth.

### 3.2. Effects of different types of microbes

Based on the results of research on the use of Streptomyces spp. give better results compared to Trichoderma harzianum and without microbes. This is indicated by the highest germination value of 90.42%.

Table 3. Average percentage of germination (%) in the treatment of Biopriming duration and types of microbes (Trichoderma harzianum and Streptomyces spp.) In soybean plants (Glycine max L.)

| Biopriming Durations (P) | Type of Microbes (M) | Average |
|--------------------------|----------------------|---------|
|                          | Control (M0)         |         |
|                          | Trichoderma harzianum (M1) |         |
|                          | Streptomyces spp. (M2) |         |
| 3 hours (P1)             | 85.00                | 88.33   | 90.00     | 87.78 |
| 6 hours (P2)             | 86.67                | 88.33   | 91.67     | 88.89 |
| 12 hours (P3)            | 83.33                | 91.67   | 86.67     | 87.22 |
| 24 hours (P4)            | 88.33                | 90.00   | 93.33     | 90.56 |
| Average                  | 85.83<sub>b</sub>    | 89.58<sub>a</sub> | **90.42<sub>a</sub>** |

This is because Streptomyces spp., Is able to produce hormones that plants need to grow. The hormone produced by the microbe Streptomyces spp., Is an IAA hormone that plays an important role for plant growth and development. This is supported by Yusepi [12] states that strains of Streptomyces spp. MBR-52 is able to accelerate the emergence and lengthening of plant adventitious roots. As well as being able to increase the germination and root length of corn and pea plants through IAA production. Isolates capable of producing IAA can be used as biological controllers through competition, antibiotic production, plant resistance induction, fit-hormone production [13,14].

### 4. Conclusions

The conclusion of this research is the treatment of immersion for 12 hours using streptomyssec spp. able to increase normal sprouts and reduce abnormal sprouts. In the single factor of immersion, no significant effect on all parameters. In the single factor type of microbial Streptomyces spp. gives the highest average value of germination.

Based on the research results, it is better for further research to use legin (pure culture of Rhizobium japonicum) on land that has never been planted with legume crops. In addition, further research is needed related to the use of seeds produced from soybean seeds that have decreased physiological quality.

### References

[1] Irwan A W 2006 Budidaya Tanaman Kedelai (Glycine max (L) Merill). Online publication http://www.wawanshut.com [Accessed on 18 January 2019].

[2] Ilyas S, Asie K V, Sutariariati G A K, Sudarsono 2015 Biomatri-conditioning or biopriming with biofungicide or biological agent applied on hot pepper (Capsicum annum L.) seeds reduced seedborne seed quality and yield Acta Hortic. 1105 89-96.
[3] Sutariati G A K, Safuan L O, Fitrianti H 2014 Uji efektifitas teknik biopriming dan sumber benih terhadap viabilitas dan vigor bibit kakao J Agriplus 24 111-122.

[4] Contreras-Cornejo H A, Macías-Rodríguez L. I., Cortés-Penagos C, López-Bucio J 2009 Trichoderma virens, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis Plant Physiol. 149 1579–1592.

[5] Olanrewaju O S, Glick B R, & Babalola O O 2017 Mechanisms of action of plant growth promoting bacteria World J of Microbiol. & Biotech. 33 197.

[6] Magda M A, Hameda E A, & Samyah D J 2012 Synergistic effect between Azotobacter vinelandii and Streptomyces sp. isolated from saline soil on seed germination and growth of wheat plant. J. American Sci. 8 667-676.

[7] Tefa A, Widajati E & Syukur M 2016 Aplikasi bakteri probiotik untuk meningkatkan mutu fisiologi dan kesehatan bibit cabai (Capsicum annuum L.) J Agronomi Indonesia (Indonesian J Agron.) 176-182.

[8] Aryantha I N P, Lestari D P & Pangesti N P D 2004 Potensi isolat bakteri penghasil IAA dalam peningkatan pertumbuhan kecambah kacang hijau pada kondisi hidroponik J. Mikrobiol. Indonesia 9 43-46.

[9] Herлина N F N & Aziz S A 2017 Peningkatan viabilitas benih jintan hitam (Nigella sativa) dengan hidropriming dan pemberian asam giberelat Bul. Pen. Tan. Rempah dan Obat 27 129-136.

[10] Copeland L O & McDonald M F 2012 Principles of Seed Science and Technology) (Germany: Springer Science & Business Media).

[11] Nurmauli & Nurmiaty Y 2010 Studi metode invigorasi pada viabilitas dua lot benih kedelai yang telah disimpan selama sembilan bulan J. ilmu Pert. Indonesia 15 20-24.

[12] Yusepi T T 2011 Kemampuan Actinomyecetes Endofit dalam Meningkatkan Pertumbuhan Tanaman Padi (Oryza sativa L.) Melalui Aktivitas Asam Indol Asetat [Theses] (Bogor: Departemen Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam IPB).

[13] Restu M, Bachtiar B and Larekeng S H 2019 Gibberellin And IAA Production by Rhizobacteria From Various Private Forest IOP Conference Series: Earth and Environmental Science vol 270 (IOP Publishing) p 12018

[14] Larekeng S H, Gusmiaty, Restu M, Tunggal A and Susilowati A 2019 Isolation and identification of rhizospheric fungus under Mahoni (Swietenia mahagoni) stands and its ability to produce IAA (Indole Acetid Acid) hormones IOP Conf. Ser. Earth Environ. Sci. 343 012051