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Seed Biopriming Using Rhizobacterial Isolated Mixture on Increasing Growth and Yield of Shallots (*Allium ascalonicum* L.)

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ABSTRACT
Shallot is very potential to be developed in Southeast Sulawesi, but its productivity is very low, so it needs technological innovation, including the use of microbes as a promoter of plant growth. This study aims to evaluate the effectiveness of shallots seed biopriming using a mixture of rhizobacterial isolates to increase the growth and yield of shallots. This research was conducted at the Experimental Garden II, Faculty of Agriculture, University of Halu Oleo. The experiment was arranged using a Randomized Block Design (RBD), consisting of 8 treatments seed biopriming using a mixture of rhizobacterial isolates, ie without rhizobacterial isolates as control, CKD061 isolate, W2R06 isolate, TWB11 isolate, CKD061+W2R06 isolate, CKD061+TWB11 isolate, W2R06+TWB11 isolate, and CKD061+TWB11+W2R06 isolate. Data were analyzed using variance analysis, followed by Honestly Significant Difference test (BNJ). The results showed that Inoculation of shallots seeds with rhizobacteria both single and mixed was more able to increase the growth and yield of shallots compared to controls. CKD061 single isolate showed better performance in increasing the growth and yield of shallots compared to other controls and isolates, but was not significantly different from mixture of CKD061 + W2R06 isolates. Increased production in the biopriming treatment of CKD061 isolates and CKD061 + W2R06 isolates respectively reached 67.69% and 53.85% compared to controls. Further testing is needed on a wider scale to get more consistent results in the field.

Keywords: *Biopriming, growth, rhizobacteria, seeds, shallots, yield*

1. INTRODUCTION

The potential for shallot development in Southeast Sulawesi is very large, considering that most of the need for shallots in this area is imported from outside regions such as South Sulawesi and East Java [1]. Until now, only a few districts have developed shallots, including North Kolaka, East Kolaka, Buton and Wakatobi, with an average productivity of only 2.02 tonnes ha^{-1} [2]. Nationally, Southeast Sulawesi only contributed 0.02% or 390 tons of the total national production of 1,580,247 tons. The land condition, which is dominated by Red-Yellow Podzolic soil with low fertility, low pH and low organic matter content, is one of the causes of the low production of shallots in this area. Therefore, various efforts need to be made to increase the carrying capacity of land in shallot cultivation, including through the use of indigenous microbes that can act as biofertilizers. The development of microbial-based biofertilizers is not only done by Indonesia, developed countries in various parts of the world have even started it earlier [3]. In addition, the increasing public awareness in consuming food products that are healthy and free from harmful chemical pesticide residues, has made biofertilizers increasingly in demand. This is certainly a very big opportunity to produce superior microbes that can be used as biofertilizers. The results of previous studies, several potential indigenous microbial isolates (rhizobacteria) from shallot rhizosphere were isolated on marginal land in

Wakatobi Regency, Southeast Sulawesi, which showed that re-application of these isolates through inoculation on shallot seeds was significantly able to increase viability and vigor. shallot seeds compared to controls [4].

In general, the bacteria isolated from the rhizosphere of the shallot plant were mainly dominated by the *Bacillus* spp., *Serratia* spp. group. and *Pseudomonas fluorescens*, [5]. This type of bacteria has advantages in addition to promote plant growth, it is also able to protect plants from various plant disease infections. Several research results indicate that rhizobacteria are able to synthesize growth hormones, including IAA, gibberellin, and cytokinins [6], [7], [8], fix nitrogen [9], [10], [11] and dissolve phosphate [6], [12]. Meanwhile, as biological controllers for pathogens, rhizobacteria are able to produce compounds or metabolites such as antibiotics, HCN, siderophores, and secrete various hydrolytic enzymes that can degrade pathogenic cells [13], [14], [15].

Each rhizobacterial isolate has a different ability in its function as a growth promoter or biological control for disease. If applied together and both are compatible, it is expected that the advantages of the two isolates will accumulate so that the effect on plants is expected to be better. This study aimed to evaluate the effectiveness of a mixture of rhizobacterial isolates on the growth and yield of shallots.

2. MATERIALS AND METHODS

The research was conducted at the Agronomy Unit of the Agrotechnology Laboratory and in the Field Laboratory II of the Faculty of Agriculture, Halu Oleo University Kendari from December 2019 to May 2020.

2.1. Experimental Design

The experiment was arranged using a randomized block design, consisting of 8 biopriming treatments of shallot seeds using a mixture of rhizobacterial isolates, namely: Control (P0), CKD061 isolate (P1), W2R06 isolate (P2), TWB11 isolate (P3), CKD061 + W2R06 isolates (P4), CKD061 + TWB11 isolates (P5), W2R06 + TWB11 isolates (P6) and CKD061 + W2R06 + TWB11 isolates (P7). Each treatment was repeated 3 times, so that there were 24 experimental units in total.

2.2. Culture of Isolate and Rhizobacterial Suspension Preparation

Culture of isolates using TSA media which has been sterilized by autoclave (T 121oC, p 1 atm, t 20 minutes). One loop of rhizobacteria was scratched quadrant on a 9 cm diameter petri dish containing TSA media, then incubated at room temperature for 48 hours. The incubated bacterial cultures were then suspended in 100 ml of sterile distilled water in a sterile bottle and in a shaker for homogenization [16]..

2.3. Seed Treatment by Biopriming of Rhizobacterial Isolates

The shallot seeds used are local Tomia seeds which have a uniform size (5-10 g per clove). Before being given the treatment, the shallot seeds were washed with running water and wiped with a tissue, then soaked in a suspension of rhizobacteria according to the treatment (seed biopriming). Biopriming of shallot seeds was carried out for 24 hours at room temperature [4].

2.4. Soil Processing and Planting

Weeds are cleaned first, then the soil is processed using a hand tractor with a depth of ± 30 cm of tillage. Furthermore, the soil was leveled to make a experimental plot with a size of 1 m x 4 m. The spacing used is 20 cm x 15 cm. Before planting the seeds, make a hole using a hole with a depth of 3 cm. After planting, the seeds are covered with organic fertilizer as much as 10 g per planting hole. Plant maintenance includes watering, weeding and controlling plant pests. The variables observed in this study were plant height, number of leaves, number of tubers and shallot production.

2.5. Data Analysis

Data were analyzed using ANOVA, followed by Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$ if the treatment showed a significant effect. All data analyzes were performed using Statistical Analysis Software.

3. RESULTS AND DISCUSSION

3.1. Plant Height

Inoculation of shallot seeds with rhizobacteria (seed biopriming) was significantly able to increase the height of shallot plants compared to controls. Seed inoculation with isolate CKD061 showed better plant high performance compared to control and other treatments, but was not significantly different from isolates W2R06 and isolates mixture CKD061 + W2R06, and W2R06 + TWB11. The increase in the height of shallots in the treatment of CKD061 isolate reached 12% compared to the control (Figure 1)

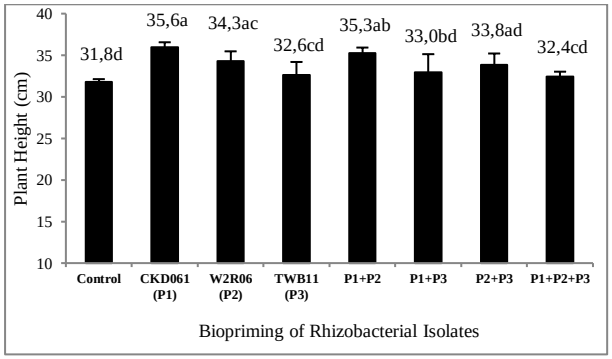

Figure 1 Effect of seed biopriming with a mixture of rhizobacterial isolates on plant height of shallot

3.2. Number of Leaves

Biopriming of shallot seeds with rhizobacteria was also significantly able to increase the number of shallots compared to control. Among the treatments tested, seed inoculation with isolate CKD061 showed better leaf count performance compared to the control and isolate mixture CKD061 + TWB11, but not significantly different from other treatments. The increase in the number of shallots in the CKD061 isolate treatment reached 28% compared to the control (Figure 2).

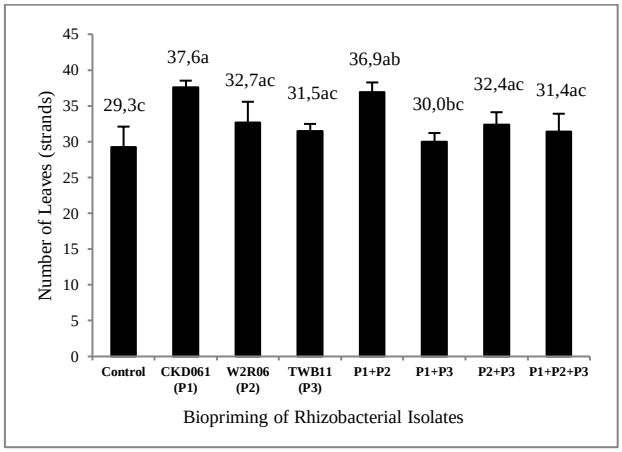

Figure 2 Effect of seed biopriming with a mixture of rhizobacterial isolates on number of leaves

3.3. Number of Tubers

Shallot seeds that were given biopriming treatment with rhizobacteria were also significantly able to increase the number of shallot tubers compared to the control. Among the treatments tested, seed inoculation with isolate CKD061 showed better leaf count performance compared to the control and isolate mixture CKD061 + TWB11, but not significantly different from other treatments. The increase in the number of shallot bulbs in the CKD061 isolate treatment reached 66% compared to the control (Figure 3).

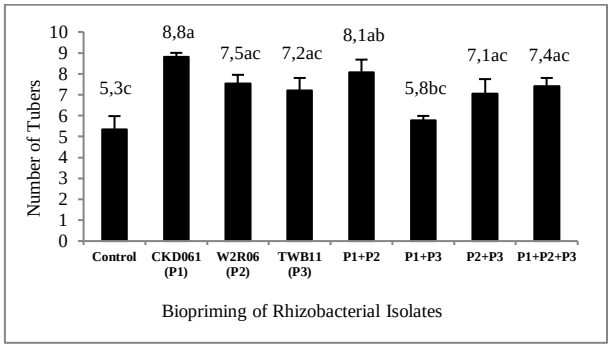

Figure 3 Effect of seed biopriming with a mixture of rhizobacterial isolates on the number of tubers

3.4. Shallot Production

Biopriming of shallot seeds with rhizobacteria was significantly able to increase shallot production compared to control. Inoculation of seeds with CKD061 isolate resulted in higher shallot production compared to control and other treatments, but not significantly different from the mixture of CKD061 + W2R06 isolates. The increase in shallot production in the CKD061 isolate treatment reached 68% compared to the control (Figure 4).

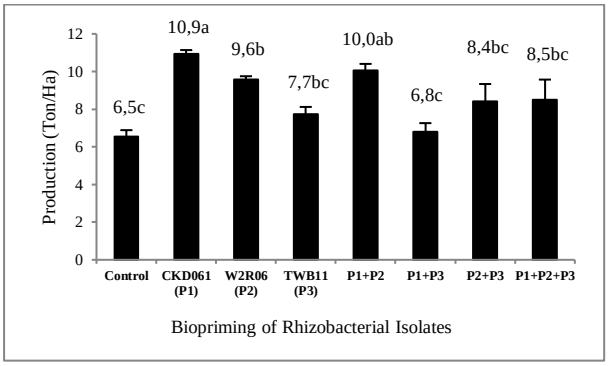

Figure 4 Effect of seed biopriming with a mixture of rhizobacterial isolates on shallot production

3.5. Discussion

Seed inoculation with indigenous rhizobacteria using biopriming techniques significantly increased the growth and yield of shallots compared to the control. The results of morphological and biochemical characterization showed that the rhizobacterial isolates CKD061 and W2R06 belonged to the Bacillus group, while TWB11 isolates belonged to the Pseudomonas group. Previous research results indicated that the three rhizobacterial isolates were proven to be able to stimulate plant growth [17], [18]. The role of rhizobacteria as plant growth promoters is related to their ability to dissolve phosphate, fix N and produce growth hormone IAA [19], [5], [20].

Several research results have proven that rhizobacteria, which are included in the PGPR (Plant Growth Promoting Rhizobacteria) group, are able to increase plant growth and yield. Both rhizobacteria from the Bacillus sp. and Pseudomonas sp. has the ability to dissolve phosphate, fix nitrogen and produce growth hormone [21], [18]. Other studies have also reported that rhizobacteria are able to increase the growth and yield of various plant commodities [22].

The results of this study indicate that the mixing of rhizobacterial isolates has not yielded the expected results. Inoculation of rhizobacteria independently actually gave better results compared to mixing. It is suspected that compatibility with one another isolates does not occur so that the excellence of each is not well expressed. This is in line with the study which states that not all mixture of isolates are able to provide a synergistic effect and give better results compared to independent applications. Among the mixture of isolates tested, only the mixture of UM96 +

UM256 isolates showed a significant increase in root length, hypocotyl length and total fresh weight of the seeds, while the mixture of other isolates had the same effect as the control [23].

4. CONCLUSION

Inoculation of shallots seeds with rhizobacteria both single and mixed was more able to increase the growth and yield of shallots compared to controls. CKD061 single isolate showed better performance in increasing the growth and yield of shallots compared to other controls and isolates, but was not significantly different from mixture of CKD061 + W2R06 isolates. Increased production in the biopriming treatment of CKD061 isolates and CKD061 + W2R06 isolates respectively reached 67.69% and 53.85% compared to controls. Further testing is needed on a wider scale to get more consistent results in the field.

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