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Increase of Seed Vigor Local Upland Rice Kambowa Cultivar Using Combination Endo-Rhizobacteria

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Abstract:- Many agricultural development technologies to increase national rice production has been carried out, one of which is the use of biological agents. Single-use of biological agents has been widely reported to increase rice production. To optimize national rice production, a combination of biological agents is applied so that rice production is expected to increase even higher. This research aimed to increase the seed vigor of the Kambowa upland rice cultivar using a combination of endo-rhizobacteria. This research was carried out at the Faculty of Agriculture, Halu Oleo University, Laboratory of Agronomy, in April 2018. The study used a completely randomized design, with 5 treatments, namely: isolates Be02 + PKLK, isolates Be02 + *Bacillus* sp. CKD061, isolates PKLK5 + *Bacillus* sp. CKD061 and isolates Be02 + PKLK5 + *Bacillus* sp. CKD061. Each treatment was repeated 3 times to obtain 15 experimental units. Variables observed included seed germination, vigor index, maximum growth potential, growth uniformity, relative growth rate, T50, and root length. The research data were analyzed using analysis of variance and continued with the DMRT$_{\alpha= 0.05}$ test. The results showed that the combination treatment of endo-rhizobacteria increased the seed vigor of the Kambowa cultivar upland rice. Combination treatments of endo-rhizobacteria isolate Be02 + isolate PKLK5 increased germination, seed vigor index, maximum growth potential, growth uniformity, and root length. Combination treatment of endo-rhizobacteria isolates Be02 + PKLK5 + *Bacillus* sp. CKD061 resulted in a relative growth rate and treatment of isolates Be02 + *Bacillus* sp. CKD061 gave the best T50 compared to the control.

Keywords:- *IAA; Isolated PKLK5; Isolated Be02; Local Upland Rice.*

I. INTRODUCTION

Rice is an important role in Indonesia because it is the major source of food for the people. The rice in Indonesia consists of various types of diversity, one of which includes upland rice. Upland rice is a local type of rice that is greatly cultivated by local people as a staple food, especially in rural areas. The availability of seed quality is the main obstacle to the upland rice cultivation system. In the past, farmer use has only used seeds obtained from other farmers or stored seeds from previous plantings with unsecured quality the seed and can result in low seed vigor. Increased seed vigor can be done by providing seed treatment through seed invigoration by utilizing environmentally friendly biological agents that can act as plant growth promotion.

Endophytic bacteria are biological agents in the form of microorganisms that can live and associate in tissues of the host plant from seed germination to advanced development that do not cause negative impacts so they can provide benefits to the host plant [1] [2]. Single-use of biological agents has been widely reported can increase plant seed germination [3] and at the same time can stimulate plant growth [4], and can increase plant resistance to pathogens

[5]. Endophytic bacteria are reportedly able to produce IAA growth hormones, gibberellin, nitrogen fixation, and phosphate soluble [6] [7] [8]. Research reported that endophytic bacteria can produce plant growth hormone which can increase plant seed germination [9] [10]. The use of endophytic bacteria can play a role in stimulating plant growth [11] and crop production [12].

Other bacteria that have the same ability to bind nitrogen, dissolve phosphates, and produce growth hormones are rhizobacteria [13] [14]. Rhizobacteria are beneficial bacteria that are commonly found in the rhizosphere, especially in the soil layer 1-2 mm around the root area. Single-use of biological agents is effective in increasing seed germination because they contain the phytohormones cytokinin and gibberellins [15] [16]. The results of research reported rhizobacterial isolates that produce IAA can increase the germination and vigor index of rice seeds [17]. That three isolates of rhizosphere bacteria, namely RC1, RC3, and RC12 were able to increase the vigor of chili seeds compared to the control [18]. Rhizobacteria are also able to stimulate growth and plant development [19] [20].

Based on the superiority of biological agents independently, it is necessary to combine endo-rhizobacteria so that they can increase the vigor of plant seeds. The results of research reported, a consortium of rhizobacteria and endophytic bacteria had a significant effect on viability and seed vigor with a germination value of more than 90% in isolates RF4, EDF1, EDF2, and EDFbt3 [21]. That bacteria live and associate well in plant tissues (endophytic bacteria) and live in the rhizosphere of plants are known to enhance growth and plant development and also manage plant diseases [19]. The result of research reported that, combined inoculation of two groups of rhizosphere bacteria and endophytic bacteria are able to increase root dry weight of 33.7% and shoot dry weight of 37.8% [22]. However, information regarding the consortium of endophytic bacteria and rhizosphere in initiating it in increasing seed vigor has not been widely reported. This research was aimed to increase the seed vigor of the Kambowa cultivar upland rice using a combination of endo-rhizobacteria.

II. MATERIAL AND METHODS

A. Place and Time

This research was carried out at the Agrotechnology Laboratory of the Agronomy Unit, Faculty of Agriculture, University of Halu Oleo, Kendari in April 2018.

B. Material and Tools

The materials used were local upland rice seeds of the Kambowa cultivar, Be02 isolate (endophytic bacterial), rhizobacterial isolates PKLK5, and *Bacillus* sp. CKD061 (collection of Prof. Dr. Ir. Gusti Ayu Kade Sutariati., M.Si), 70% alcohol, Trypthic Soy Broth (TSB), agar, spirits, tissue, plastic wrap, label paper and charcoal husk. The tools used in this study were trays, hand sprayers, analytical scales, ose needles, petridish, bunsen, measuring cups, scott bottles, shakers, stirrers, autoclave, laminar air flow cabinet (LAFC), erlenmeyer, beakers glass, cameras, ruler and writing utensil.

C. Eksperimental Design

The study used a completely randomized design (CRD) with 5 treatments, namely**:** control, Be02 + PKLK5 isolate, Be02 isolate + *Bacillus* sp. CKD061, PKLK5 isolate + *Bacillus* sp. CKD061 and Be02 + PKLK5 isolate + *Bacillus* sp. CKD061, each treatment was repeated three times to obtained 15 experimental units.

D. Isolate Propagation and Seed Treatments

Microbial isolates were propagated with TSA media, weighing 30 g of TSB and 20 g of agar, then dissolved in 1000 ml of distilled water and boiled for ± 20 minutes. After boiling, put the media into Scott's bottles and sterilized using an autoclave (T 121ºC, p 1 atm, t 20 minutes). Then the TSA media was poured into a petri dish with a thickness of ± 0.5 cm aseptically in LAFC and then cooled and ready to use. Furthermore, isolated of bacterial endo-rhizobacteria was grown on solid TSA media and incubated for 48 hours. The growing bacterial colonies were suspended in sterile distilled water until they reached a population density of 10^9 cfu ml^{-1}.

After the suspension of endophytic bacteria and rhizobacteria was ready, the local upland rice cultivars of Kambowa (seeds with a shelf life of 3 years) were disinfected with 5% sodium hypochlorite for 5 minutes, then washed with sterile water 3 times and then air-dried in LAFC. The local upland rice seeds were then put into the bacterial suspension according to the treatment and for the control only soaked using sterile distilled water and incubated for ± 24 hours. After treatment, the seeds were again air-dried again in LAFC. Furthermore, seed viability and vigor tests were carried out, that is 25 seeds were germinated in trays containing sterile husk charcoal.

E. Observations Variable

Observations were made on the variables of seed viability and vigor as well as sprout root length, as follows:

- **Seed Germination (%)**. Seed Germination (SG) describes the potential viability of seeds [23], calculated based on the percentage of normal seedling (NS) on the last day of observation (7 day after planting (dap)) with the formula:

$$SG = \frac{\text{Total of number normal seedlings}}{\text{Total of number seeds planted}} \times 100 \quad (1)$$

- **Maximum Growth Potential (%).** Maximum growth potential (MGP) describes the total viability of seeds [23], 1999), calculated based on the number of seeds germinating:

$$MGP = \frac{\text{Total of number seeds germinated}}{\text{Total of number seeds planted}} \times 100 \quad (2)$$

- **Growth Uniformity (%).** Growth uniformity (GU) describe the seed vigor, calculate based on the percentage of normal seedling (NS) on the day between the first count (5 dap) and the second (7 dap) i.e. at 6 dap, with the formula:

$$GU = \frac{\sum \text{NS between at observation 1 \& 2}}{\text{Total of number seeds planted}} \text{ X } 100 \quad (3)$$

- **Vigor Index (%).** Vigor index (VI) calculate based on the percentage of normal seedling (NS) on the day between the first count. observed on the 5th day was calculated by the formula:

$$VI = \frac{\sum \text{NS at observation 1}}{\text{Total of number seeds planted}} \text{ X } 100 \quad (4)$$

- **Relative Growth Rate (% etmal^{-1}).** Relative growth rate (RG^R), calculate based on the accumulated growth rate every day by observing the percentage of normal seedling per day. In rice seeds, the relative growth rate is calculated by comparing the RG value with the maximum RG obtained from the assumption that when the growth count of normal seedling reaches 100%.

$$GR = \sum_{0}^{n} \frac{N}{t} \qquad RG^R = \frac{GR}{\text{Max. GR}} \text{ X } 100\%$$

$$\text{Max. RG} = \frac{100}{\sum \text{NS at observation 1}} = \frac{100}{5} = 20 \quad (5)$$

Note:
GR = Growth rate
t = times of observation
N = percentage of normal seedling at each observation time

- **T_{50} (day).** T_{50} is the time required to reach 50% total seedling germinate, observations are made every day. The T50 formula ie.:

$$T_{50} = ti + \left(\frac{n50\% - ni}{nj - ni} \right) \quad (6)$$

Note:
T_{50} = Time before seed germination 50%
ti = After time of seed germination 50%
n50 = The amount of seed germination 50% of the total seed germination
ni = The amount of seed germination at the time after seed germination 50%
nj = The amount of seed germination at the time before seed germination 50%.

- **Root length (cm)**. Observations were carried out by measuring the length of the sprouting root using a ruler.

F. Data Analysis

Observational data for each treatment were tabulated and followed by an analysis of variance. The results of the analysis of variance which showed a significant effect were followed by the DMRT test $\alpha = 0.05$.

III. RESULT

The results showed that the combination application treatment of endo-rhizobacteria had a significant effect on seed viability and vigor. The results of test $DMRT_{\alpha=0.05}$ combination treatment of endo-rhizobacteria on the observation of seed germination and vigor index; maximum growth potential and seed uniformity; relative growth rate and T50 as well as root length are presented in Table I, Table II, Table III and Table IV.

The results in Table I show that the highest treatment for germination was obtained in the combination of Be02 + PKLK5 isolates at 89.33% (19.64% increase in treatment), which was not significantly different from the combination of Be02 + PKLK5 isolates + *Bacillus* sp. CKD061 was 84.00% (12.49% increase in treatment) but significantly different from other treatments, especially without treatment which only reached 74.67%.

TABLE I. AVERAGES OF GERMINATION AND SEED VIGOR INDEX OF LOCAL UPLAND RICE CULTIVARS OF KAMBOWA IN COMBINED TREATMENT WITH ENDO-RHIZOBACTERIA (± SE (STANDAR ERROR))

Treatments	Seed Germination(%)	Vigor Index (%
Control	74.67 ± 2.31 ^{b}	65.33 ± 2.31 ^{c}
Isolates Be02 + PKLK5	89.33 ± 4.62 ^{a}	84.00 ± 4.00 ^{a}
Isolates Be02 + *Bacillus* sp. CKD061	80.00 ± 4.00 ^{b}	74.67 ± 6.11 ^{b}
Isolates PKLK5 + *Bacillus* sp. CKD061	76.00 ± 6.93 ^{b}	70.67 ± 4.62 ^{bc}
Isolates Be02 + PKLK5 + *Bacillus* sp. CKD061	84.00 ± 4.00 ^{ab}	73.33 ± 4.62 ^{bc}

Note: The numbers followed by different letters in the same column are significantly different based on the $DMRT_{\alpha= 0.05}$ test

TABLE II. AVERAGES OF MAXIMUM GROWTH POTENTIAL AND GROWTH UNIFORMITY OF LOCAL UPLAND RICE CULTIVARS OF KAMBOWA IN COMBINED TREATMENT WITH ENDO-RHIZOBACTERIA (± SE)

Treatments	Maximum Growth Potential (%)	Growth Uniformity (%)
Control	81.33 ± 4.00 ^{b}	72.00 ± 6.93 ^{b}
Isolates Be02 + PKLK5	92.00 ± 8.33 ^{a}	86.67 ± 6.93 ^{a}
Isolates Be02 + *Bacillus* sp. CKD061	86.67 ± 0.00 ^{ab}	77.33 ± 6.93 ^{b}
Isolates PKLK5 + *Bacillus* sp. CKD061	86.67 ± 9.24 ^{ab}	74.67 ± 6.11 ^{b}
Isolates Be02 + PKLK5 + *Bacillus* sp. CKD061	90.67 ± 4.620 ^{a}	76.00 ± 4.62 ^{b}

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Note: The numbers followed by different letters in the same column are significantly different based on the $DMRT_{\alpha=0.05}$ test

The results of the study in Table II show that the highest treatment for maximum growth potential was obtained in the combination of Be02 + PKLK5 isolates of 92.00% with an increase of 13.24% which was not significantly different from the other treatments but significantly different from the control only by 81.33 %.

TABLE III. AVERAGES OF RELATIVE GROWTH RATE AND T_{50} OF LOCAL UPLAND RICE CULTIVARS OF KAMBOWA IN COMBINED TREATMENT WITH ENDO-RHIZOBACTERIA (± SE)

Treatments	Relative Growth Rate (% etmal^{-1})	T50 (days)
Control	77.57 ± 1.41 ^{b}	3.31 ± 0.36 ^{a}
Isolates Be02 + PKLK5	89.84 ± 2.21 ^{a}	2.41 ± 0.21 ^{b}
Isolates Be02 + *Bacillus* sp. CKD061	89.02 ± 1.43 ^{a}	2.09 ± 0.09 ^{b}
Isolates PKLK5 + *Bacillus* sp. CKD061	80.73 ± 2.24 ^{b}	2.45 ± 0.31 ^{b}
Isolates Be02 + PKLK5 + *Bacillus* sp. CKD061	89.49 ± 2.94 ^{a}	2.43 ± 0.28 ^{b}

Note: The numbers followed by different letters in the same column are significantly different based on the $DMRT_{\alpha=0.05}$ test

The results of the observations in Table III show that the highest relative growth speed was obtained in the treatment of Be02 isolates + PKLK5 isolates + *Bacillus* sp. CKD061 was 89.49% etmal^{-1} (15.37% increase), which was not significantly different from the combination treatment of isolates Be02 + PKLK5 and isolates Be02 + *Bacillus* sp. CKD061 (14.76% increase) but significantly different from other treatments, especially the control treatment, only 77.57% etmal-1. The results of observations of the fastest T50 were obtained in the treatment of isolates Be02 + *Bacillus* sp. CKD061 for 2.09 days (37.00% increase), which was not significantly different from the other treatments, but significantly different from the control for 3.32 days.

TABLE IV. AVERAGES OF ROOT LENGTH OF LOCAL UPLAND RICE CULTIVARS OF KAMBOWA IN COMBINED TREATMENT WITH ENDO-RHIZOBACTERIA (± SE)

Treatments	Root Length (cm)
Control	8.98 ± 0.71 ^{b}
Isolates Be02 + PKLK5	12.82 ± 0.50 ^{a}
Isolates Be02 + *Bacillus* sp. CKD061	12.72 ± 0.71 ^{a}
Isolates PKLK5 + *Bacillus* sp. CKD061	12.60 ± 0.96 ^{a}
Isolates Be02 + PKLK5 + *Bacillus* sp. CKD061	12.44 ± 0.83 ^{a}

Note: The numbers followed by different letters in the same column are significantly different based on the $DMRT_{\alpha=0.05}$ test

The results of the $DMRT_{\alpha=0.05}$ test in Table IV show that the longest roots was obtained in the treatment of isolate Be02 + *Bacillus* sp. CKD061 of 12.82 cm which was not significantly different from the other treatments but significantly different from the control of 8.98 cm. This shows that the treatment of the endo-rhizobacteria consortium was able to increase the root length of local upland rice plants.

IV. DISCUSSION

The results showed that the combination application treatment of endo-rhizobacteria had a significant effect on seed viability, and vigor and the root length of local upland rice. The results of observations on the highest seed vigor index were obtained in the treatment of Be02 isolates + *Bacillus* sp. CKD061 (B) of 84.00% (24.58% treatment effectiveness), which was significantly different from other treatments, especially the control of 65.33%. This is because the use of endo-rhizobacteria bacteria can produce the growth hormone indole acetic acid (IAA) which functions to boost germination and vigor index. The results of research reported that, a consortium of rhizosphere and endophytic bacteria had a significant effect on seed viability and vigor with germination values of more than 90% in isolates RF4, EDF1, EDF2, and EDFbt3 [21]. A consortium of *Rhizobium leguminosarum* RPN5 and *Pseudomonas* sp. PPR8 showed a 65% increase in seed germination and emergence as compared to the control [24]. Furthermore, the highest vigor index was obtained by using double inoculants *R. leguminosarum* RPN5 and *Bacillus* sp. BPN-7 and the lowest in the control treatment or those not inoculated on ordinary peanuts. Other research also reported an increase in strength index with the use of *Trichoderma viridae* and *Rhizobium* sp. [25].

The results of the research on the observation of the highest growth simultaneity were obtained in the Be02 + PKLK5 isolate treatment of 86.67% with an increase of 20.37% which was significantly different from other treatments, especially the control only reaching 72.00%. Endophytic bacteria and rhizobacteria have been reported to be able to produce growth hormones in the form of IAA [8] 13] and gibberellin [6] [26]. The results of research reported, that sixteen rhizosphere bacterial isolates were able to produce the phytohormone IAA in the range of 7.96-47.23 ppm which could be useful in increasing seed germination [18]. Other research also reported that endophytic bacteria can produce plant growth hormone which can increase plant seed germination [6] [9] [10]. Direct treatment of biological agents by modifying the microflora around the seeds can increase seed vigor [27] [28]. In line with the result of research reported, endo-rhizobacteria consortium isolate Be02 + PKLK5 + *Bacillus* sp. CKD061 can increase germination (25.49%), growth uniformity (18.75%), and maximum growth potential (26.32%) when compared to the control [29].

The results of this study indicate that the treatment of biological agents can accelerate seed germination for the parameters of relative growth speed and T50. According the

result of research reported that, endophytic-rhizobacterial isolates L1-R, LA6-R and LA2-E were able to increase the vigor and viability of areca nut seeds compared to control isolates because of their ability to produce growth hormone IAA which plays an important role in the process of seed germination [8]. This is relevant to the research reported, bacteria that produce gibberellins and IAA can stimulate seed germination and stimulate shoot growth [12]. The phytohormone IAA can suppress abscisic acid (ABA), which is an inhibitory compound in germination so that the germination process can take place quickly and optimally [30].

The results showed that the combination treatment of endo-rhizobacteria effectively increased the root length of upland rice seed sprouts with the highest increase of 42.76% obtained in the treatment of isolate Be02 + *Bacillus* sp. CKD061 when compared to control. Seed germination is a complex biological process, that is regulators controlled by a variety of, including phytohormones such as auxin and gibberellin. This hormone will stimulate the process of seed germination so that it can bring up roots and shoots. IAA is an endogenous auxin that plays an important role in cell expansion, root development, and elongation, even can stimulate water absorption so that the imbibition process in seeds is fast due to germination can take place well in forming plant organs such as roots and leaves [31]. Furthermore reported, explained that the role of IAA is produced exogenously from bacteria can accelerate plant growth by spurring the differentiation of processes in roots in forming root hairs [18]. Also reported that the use of a consortium of biological agents isolates DM and K1K1 has the effect of increasing the formation of lateral roots and as a potential source of bioactive metabolites that could even increase root length, shoots, and root dry weight [32]. Application of *A. lipoferum* increased root length, root dry weight, and shoot dry weight up to 30%, 50%, and 34% respectively [33].

V. CONCLUTION

Combination treatment of endo-rhizobacteria isolates Be02 + isolate PKLK5 increased germination, seed vigor index, maximum growth potential, growth uniformity, and root length. Combination treatment of endo-rhizobacteria isolates Be02 + PKLK5 + *Bacillus* sp. CKD061 resulted in a relative growth rate and treatment of isolated Be02 + *Bacillus* sp. CKD061 gave the best T50 compared to the control.

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