Journal of Biological Science and Education
~JBSE~
Websites:http://usnsj.com/index.php/biology
E-mail:biologi_jbse@usn.ac.id

Response of Cocoa Seed Growth Received by Bio-priming Technique Treatment

AUTHOR INFO
Fitrianti Handayani
Universitas Sembilanbelas November Kolaka
Fitriantihandayani87@gmail.com
+62822223431184

Mustafa R
Universitas Sembilanbelas November Kolaka
Mustafamp@yahoo.co.id
+6282344383978

Maretik
Universitas Sembilanbelas November Kolaka
Maretik237@gmail.com
+6285210100688

Rosmawati
Universitas Sembilanbelas November Kolaka

ARTICLE INFO
E-ISSN: 2721-0804
P-ISSN: 2723-6838
Vol. 3, No. 2, December 2021
URL: http://usnsj.com/index.php/biology

Suggestion for the Citation and Bibliography
Citation in Text:
Handayani, et al. (2021)
Bibliography:
Handayani, F., Mustafa, Maretik, & Rosmawati. (2021). Response of Cocoa Seed Growth Received by Bio-priming Technique Treatment. *Journal of Biological Science and Education, 3*(2), 64-72.

Abstract

Cocoa (*Theobroma cacao* L.) is one of the people's plantation crops with promising prospects because it can flower and bear fruit throughout the year. One of the efforts to improve the quality of cocoa seeds is by increasing the viability and vigor of seeds through seed invigoration techniques. This study aimed to determine the effect of biopriming invigoration treatment techniques in increasing cocoa seeds' viability, vigor, and growth. This research was conducted at the Laboratory of Agronomy Unit of the Faculty of Agriculture of Halu Oleo Kendari from April to May 2019. This study used a completely randomized design (CRD) consisting of 4 treatments: Control (B0), Bio-priming Bacillus sp. CKD061 (B1), Bio-priming Pseudomonas flourescent (B2), a combination of Bio-priming Bacillus sp. CKD061 with Pseudomonas fluorescent. Each treatment was repeated three times, so there were 12 experimental units. The data obtained were analyzed using variance analysis and continued using the Least Significant Difference Test (LSD). Based on the results of research that biopriming treatment using rhizobacteria has the best influence on the viability, vigor and growth of cocoa seeds.

Keywords: Biopriming, cocoa seeds, rhizobacteria, seed growth

A. Introduction

Cocoa (*Theobroma cacao* L.) is one of the people's plantation crops with promising prospects because it can flower and bear fruit throughout the year to become weekly and even daily income for farmers. Cocoa production is currently 435,000 tons, with production from smallholder plantations at around 87%. The highest production, 67%, was obtained from cocoa production centre areas centred in South Sulawesi, Southeast Sulawesi, and Central Sulawesi (Maemunah, 2009; Handayani, 2014).

The potential land for plantations in Southeast Sulawesi is 246,508 ha, which has just been utilized for 180,941 ha with a production of around 146,705 tons, which are generally people's plantations that are managed intensively (Plantation and Horticulture Office, 2012). However, cocoa productivity has decreased because the cocoa plants are old. According to Suhendy, 2007), cocoa plants that are 25 years old are only half of their production potential.

Some of the factors that cause a decrease in cocoa quality productivity are the age of the old plant. Deficient cultivation techniques also impact high pest and disease attacks (Sutariati et al., 2014). And the lack of maintenance carried out by planters (Directorate General of Plantations, Ministry of Agriculture, 2010).

Efforts that can be made to increase cocoa productivity are rejuvenation, plant rehabilitation by using quality seeds. One of the efforts to improve the quality of cocoa seeds is by increasing the viability and vigor of seeds through seed invigoration techniques.

The invigoration technique is a process that is carried out to increase the vigor of seeds that have experienced setbacks. Invigoration is an alternative to overcome low seed quality by balancing the water potential of the seed to stimulate metabolism in the seed so that the seed is ready to germinate. The application of invigoration treatment must pay attention to several things, including the type of seed, duration of incubation, level of seed vigor during treatment, drying of seeds after hydration and certain chemicals.

Seed priming treatment that is integrated with rhizobacteria is called biopriming. The results showed that rhizobacteria from the Bacillus spp., Pseudomonas spp. group, isolated from healthy chilli and tomato plants, could produce the growth hormone IAA (Sutariati et al. 2012). B. polymixa BG25, S. liquefaciens SG01, and P. fluorescent PG01 can increase seed vigor and growth of chili plants (Sutariati et al. 2006; Sutariati and Wahab 2011) and sorghum plants (Sutariati & Khaeruni 2013). Bacillus spp. able to synthesize IAA (ElSorra et al. 2007). P. flourescens can also produce IAA (Ashrafuzzaman et al. 2009). Bacillus and P. fluorescens were able to grow and develop both in number and activity after cloning maize roots (Kapli et al., 2017). Bacillus spp., Pseudomonas spp., can benefit plant growth (Tilak et al., 2005).

B. Literature Review

1. *Characteristics of Cocoa Plants*

Cocoa beans are epigeous, which means that the elongated hypocotyl lifts the cotyledons that are still closed above the soil surface. This phase is the first phase and is often referred to as the soldier phase. At the beginning of cacao seed germination, the taproot grows rapidly at the age of one week with a length of 1 cm, then at the age of one month, the taproot reaches 16-18 cm, at the age of three months, the root length is 25 cm. After that, the growth rate decreases, and it takes two years to reach 50 cm in length. Then the second phase is marked by the opening of the cotyledons, the elongation of the epicotyl and the growth of the first four leaves. The four leaves grow from each internode, but the nodes are so short that they appear to only grow in one internode.

The growth of cocoa stems is dimorphic which has two types of vegetative growth, namely orthotropic branches (growing upwards) and plagiotropic branches (growing sideways). Cocoa plants are propagated by seeds and will form the main stem before growing primary branches. Primary branches grow to an ideal height of 1.2 – 1.5 m from the ground and cannot be propagated vegetatively.

Cocoa leaves have two joints or cartilages attached to the base and petiole. The petiole is finely scaly and forms an angle of 30-60-0and cylindrical. The colour of the young leaves is reddish to red depending on the variety (Siregar et al., 2008). The leaves are also dimorphic. The petiole length in orthotropic shoots is 7.5-10 cm, while in plagiotropic nodes, the petiole length is only 2.5 cm. The petiole is cylindrical and finely scaly, depending on the type (Center for Plantation Research and Development, 2010).

Cocoa flowering is called cauliflora and ramiflora, because the flowers and fruit grow attached to the stem or branch, where the flowers are only present until the secondary branch. Cocoa flowers are perfect flowers, consisting of 5 petals (calyx) and 10 stamens (Androecium). The flower diameter is 1.5 cm. Flowers are supported by flower stalks that are 2-4 cm long. The number of cacao flowers in one tree reaches between 5000-12000 flowers in one year. However, the number of mature flowers produced is only 1% (Siregar et al. 2008). Cocoa flowers grow in groups on flower pads attached to old stems, branches or twigs. The bearings on the flower-growing branches are called ramiflora, and those on the flower-growing stems are called cauliflora. Pollen is only 2-3 microns in diameter (Sugiharti, 2006).

According to the variety, cocoa pods have different shapes, sizes, and colours are about 10-30 cm long. The fruit's skin has 10 grooves and is 1-2 cm thick, has very soft seed flesh (Indonesian Coffee and Cocoa Research Center, 2010).

2. Cocoa Growing Conditions

Climate

The climatic conditions that are suitable for cocoa plants include: sufficient and evenly distributed rainfall, with an amount of rainfall 1500-2500 mm/year with a dry month of no more than three months, an average temperature between 15-300C, with an optimum temperature of 25, 50C, daily temperature fluctuations are not more than 90C, and there is no strong wind (Directorate General of Plantations, Ministry of Agriculture, 2010).

Soil

Soil suitable for cocoa plants has a clay loam texture, which is a combination of 50% sand, 10-20% silt, and 30-40% clay. This soil texture can hold high moisture and has good air circulation. The suitable soil texture for cocoa plants is sandy clay and sandy loam (Susanto, 1997).

3. Seed Propagation

Generative Propagation

Cocoa plants can be propagated generatively and vegetatively. For generative propagation, the recommended planting material is superior F1 hybrid seeds. The criteria for excellence include high productivity (> 2 tons/ha/year), good yield quality (seed weight > 1 g), and tolerance to major pests and diseases (Phytophthora palmivora and Helopeltis). Proper seed propagation and management can produce superior seeds (Directorate of Spice and Refreshment Plants, 2009).

Vegetative Propagation

Vegetative propagation of cacao plants has long been carried out on noble cocoa plants by grafting and using planting material in the form of entries of superior clones of the types DR 1, DR 2, and DR 3. Vegetative propagation by grafting can be carried out on lindak cocoa plants by using planting material in superior lindak cocoa clones (Center for Indonesian Coffee and Cocoa Research, 2010).

4. Seed invigoration technique

Invigoration or priming of seeds can be done through hydropriming, which soaking the seeds using a certain solution (Hatta, 2009). Seed invigoration treatment is carried out to overcome the low productivity caused by low vigor seeds. One of the invigorating treatments is seed priming or seed conditioning to accelerate and uniform growth and increase the percentage of sprouts and seedlings appearing (Wahid et al., 2008; Moradi and Younesi, 2009). The seed invigoration technique is proven to increase the viability and vigor of plant seeds (Wahid et al., 2008; Moradi, 2009).

The seed invigoration technique integrated with rhizobacteria can improve chilli seeds' physiological (viability and vigor) and pathological (health) quality. Rhizobacteria Bacillus polymixa BG25, which was integrated with sawdust matrix conditioning, effectively improved the quality of chili seeds (Sutariati et al., 2009a) and was able to increase the viability and vigor of soybean seeds (Sutariati et al., 2009b). Several genera of Bacillus such as B. subtilis, B. cereus, B. licheniformis, B. megaterium, and B. pumilus can act as biocontrol agents to control the growth of Fusarium sp (El-Hamshary and Khattab, 2008).

C. Methodology

1. Research Design

The research was carried out at the Agrotechnology Laboratory of the Agronomy Unit, Halu Oleo Kendari Faculty of Agriculture, which was held from April to May 2019. The materials used in this study were cocoa seeds, aquades, isolates of indigenous bacteria Bacillus sp. CKD061, P. fluorescens, (isolate collection of Prof. Dr. Ir. Gusti Ayu Kade Sutariati, M.Si), tissue, aluminium foil, spirit, 70% alcohol, Tryptone Soya Agar (TSA), label. The tools used in this research are a gembor, oven, analytical balance, ose needle, petri dish, Bunsen lamp, autoclave, laminar airflow cabinet, measuring cup, hot plate, etc, erlenmeyer, stirrer, camera, and writing instruments.

The research design used was a completely randomized design (CRD) to test the viability of cocoa seeds that received seed bio-invigoration treatment in a single factor pattern consisting of 4 (four), namely: Control (B0), Bio-priming Bacillus sp. CKD061 (B1), Bio-priming P. Fluorescens PG01 (B2), Bio-priming Bacillus sp. + P. Fluorescens (B3). All treatments were repeated 3 (three) times, so there were 12 experimental units.

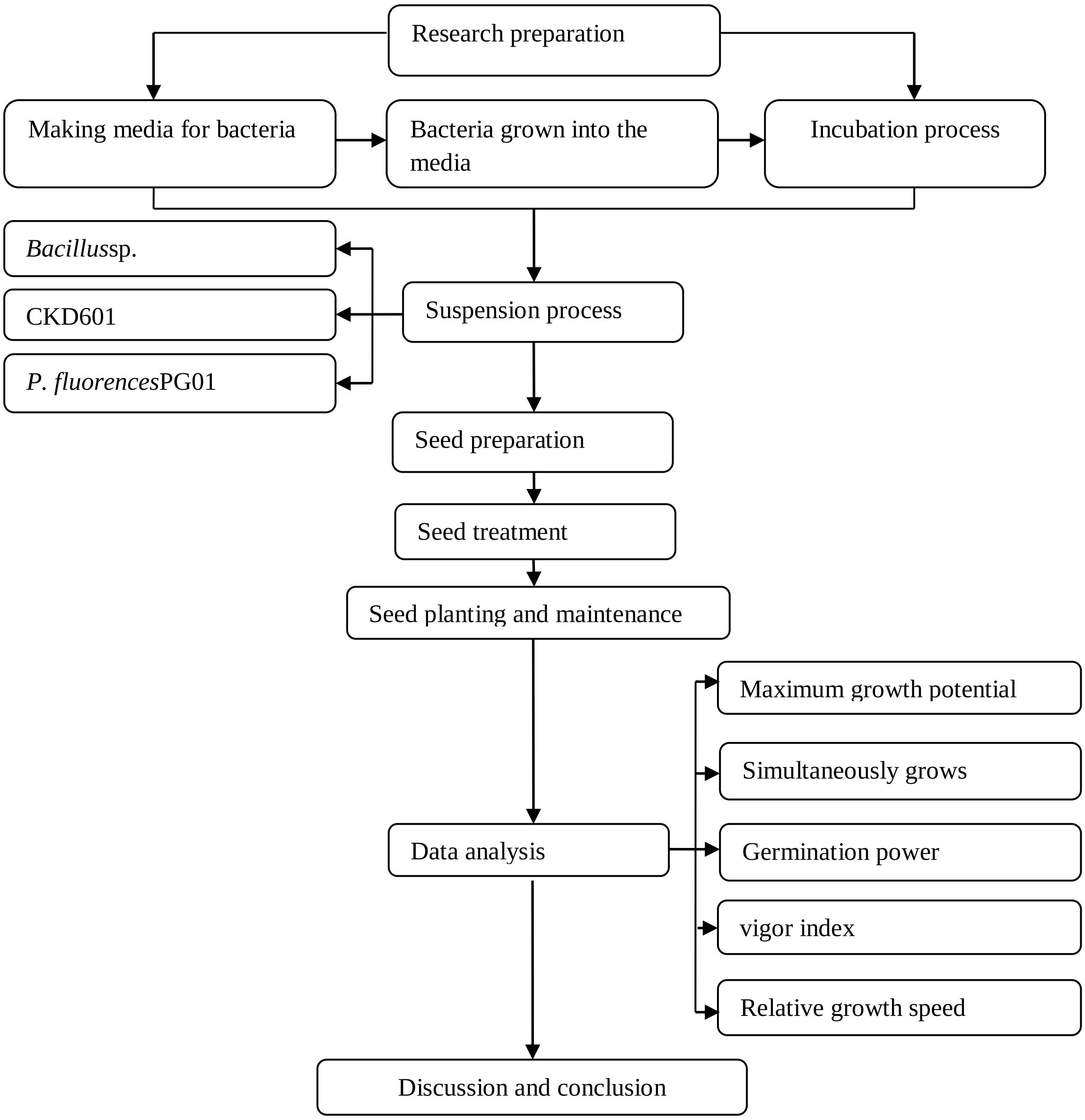

Figure 1. Research Flow

2. *Instruments*

a. *Preparation and Propagation of Rhizobacteria Isolates*

The medium used for bacterial propagation is TSA. They make TSA media 20 g dissolved in 500 ml of distilled water and boiled until boiling for±20 minutes. The mixture of boiling materials was put into an Erlenmeyer and sterilized using an autoclave (T 121 0C, t 20 minutes). Then the mix of materials is poured into a petri dish in a laminar airflow cabinet, then cooled and ready to use.

Rhizobacteria, before use, were first grown in TSA solid media (Bacillus sp. CKD061 and Pseudomonas fluorescens PG01) and incubated for 48 hours. Bacterial colonies that grow are suspended in sterile distilled water

Suspension Preparation

The suspension that will be used in this study is a suspension of Bacillus sp. CKD601, P. fluorencens PG01 *b. Seed Preparation*

The seeds used in this research are physiologically ripe cocoa seeds. The characteristics of cocoa seeds that have reached physiological maturity are changes in fruit colour: the fruit, when ripe, is green or red, and when physiologically mature, it changes colour to yellow-green and yellow-orange.

c. Seed Treatment

The cacao bean slime is opened using rubbing ash, then the seed coat is removed, then the seeds are immersed in a suspension of Bacillus sp. CKD061, suspension of an isolate of P. fluorescens PG01, suspension of an isolate of Bacillus sp. CKD061 + P. fluorescens for 5 hours.

d. Seed Planting and Maintenance

Seeds were planted in a germination box of 20 seeds for each treatment. Maintenance was carried out during the research, namely watering two times a day (morning and evening) and adjusted to the humidity of the growing media.

3. Technique of Data Analysis

Observations were made on the viability and vigor of the seeds. Observations on seed viability were maximum growth potential (PTM) and germination capacity (DB). Observations on seed vigor were simultaneous growth (KST), vigor index (IV), and relative growth speed (Kct-R).

Maximum Growth Potential (%)

Maximum Growth Potential (PTM) (%), is the total viability, which describes the total live seeds. PTM is calculated based on the percentage of seeds growing either normal or abnormal:

$$\text{PTM} = \frac{\Sigma\ Growing\ seeds}{\Sigma\ Planted\ seeds} \text{x } 100\%$$

Germination (%)

Germination Power (DB) (%) is one of the potential viability parameters expressed in percent. DB is calculated based on the percentage of normal germination (KN) in the first and second observations, or if the counts I and II have not been determined, calculated based on the total KN at the end of the observation:

$$\text{DB} = \frac{\Sigma\ KN\ count\ I + \Sigma\ KN\ count\ II}{\Sigma\ Planted\ seeds} \text{x } 100\%$$

Simultaneous Growth (%)

Simultaneous Growth (KST) (%), can be calculated based on the percentage of normal and even germination on the 10th day (the day between count I and count II of the germination test).

$$\text{KST} = \text{x } 100\% \frac{\Sigma\ Normal\ sprouts}{\Sigma\ Planted\ seeds}$$

Vigor Index (%)

Vigor Index (IV) (%), describes the growth speed vigor, calculated based on the percentage of normal sprouts on the first count with the formula:

$$\text{IV} = \text{x } 100\% \frac{\Sigma\ KN\ count\ I}{\Sigma\ Planted\ seeds}$$

Relative Growth Speed (%/etmal)

Relative Growth Speed (Kct-R) (%/etmal), describing seed vigor, is the ratio of the Kct value to the maximum Kct. The maximum Kct itself was obtained from the assumption that the normal sprouts had reached 100% at the first count. Kct is calculated based on the accumulated daily growth rate with the formula:

$$\text{KCT-R} = \frac{\text{KCT}}{\text{KCT max}}$$

$$KCT = \sum_{0}^{tn} \frac{N}{t}$$

Information :

T = observation time

N = % KN each observation time

mr = end time of observation

$$KCT\ max = \frac{100}{\Sigma\ day\ count\ I}$$

Data were analyzed by using variance fingerprint. If there is a significant effect in the variance, proceed with the Least Significant Difference Test (BNT) at the 95% confidence level.

D. Findings and Discussion

1. *Findings*

Maximum growth potential (%)

The average maximum growth potential of cacao seeds has no significant effect.

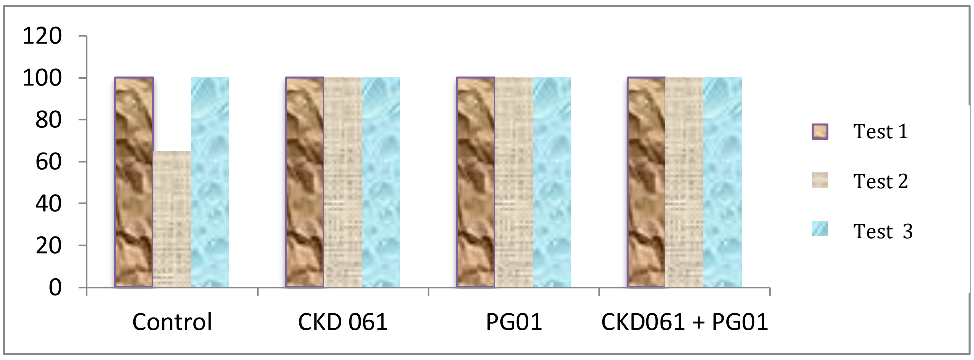

Figure 2. Maximum growth potential. Description of treatment Control (without treatment), CKD061 = Bacillus sp, PG01 = Pseudomonas fluorescent, and CKD061+PG01 = a combination of Bacillus sp with Pseudomonas fluorescent.

Based on the results of observations made that the maximum growth potential apart from control in the 2nd replication which only had the growth potential of 65% of all treatments (Control u1, Control u3, CKD061 u1, CKD061 u2, CKD061 u3, PG01 u1, PG01 u2, PG01 u3, CKD01+PG01 u1, CKD061+PG01 u2 and CKD061 PG01 u3 have 100% growth potential.

Germination power

The results of the analysis of the variety of germination power of cocoa seeds have no significant effect; the average germination of cocoa seeds is shown in Figure 3.

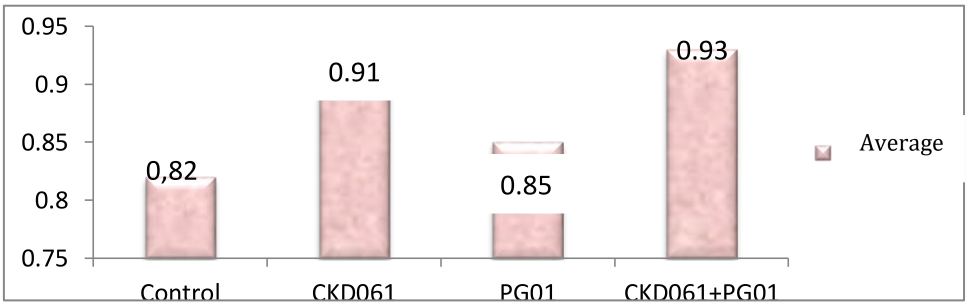

Figure 3. Germination of cacao seeds.

Based on the results of observations on the average germination of cocoa seeds, the highest germination rate was in the combination treatment of rhizobacteria Bacillus CKD061 with Pseudomonas fluorescent with a germination rate of 0.93, then Bacillus CKD061 treatment had a germination rate of 0.91 followed by Pseudomonas fluorescent PG01 treatment. with germination of 0.85 and the treatment that had the lowest germination was found in the control (without treatment) which had a germination percentage of 0.82.

Simultaneously grows

The results of the analysis of the variance of the simultaneous growth of cocoa seeds did not have a significant effect. The average uniformity of growth is shown in Figure 4.

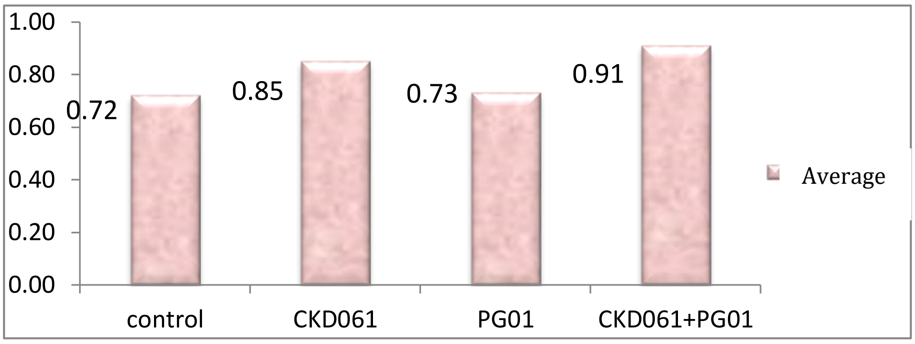

Figure 4. Simultaneity grows.

Based on the observation results of the average simultaneous growth of cocoa seeds, the highest percentage of simultaneous growth was in the combination treatment between Bacilus sp CKD061 and Pseudomonas fluorescent, which was 0.91, then Bacillus sp CKD061 0.85, and the lowest yield was found in control (without treatment).

Vigor Index

The results of the analysis of the vigor index are in appendix 5a and 5b, where the vigor index has a significant effect.

Table 1.vigor index. Note: The same letter beside the value indicates no significant difference, while the different letter besides the value indicates a significant difference at the 0.05 BNT test level.

Treatment	Vigor Index	NP 0.05
Control	0.31b	
CKD061	0.54a	0.145
PG01	0.50a	
CDK061+PG01	0.51a	

Based on the observation that from the four treatments of Bacilus sp, Pseudomonas fluorescent, and a combination of Bacillus sp. and Pseudomonas fluorescent were not significantly different, but Bacillus sp., Pseufomonas fluoerescent, and a combination of Bacillus sp. and Pseudomonas fluorescent was significantly different from the control.

Relative Growth Speed (%/etmal)

The analysis of variance in relative growth rates did not have a significant effect.

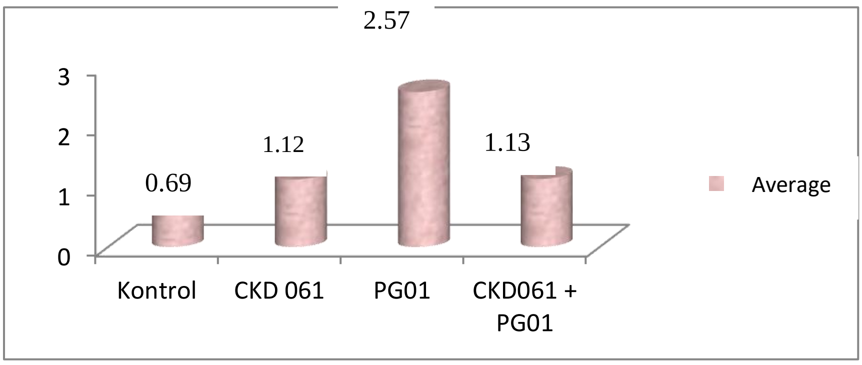

Figure 5. Relative growth speed.

Based on the results of observations of the average relative growth speed of cocoa seeds, the highest average was found in the Pseudomonas fluorescent treatment which was 2.57% / etmal, then the combination treatment between Bacillus sp and Pseudomonas fluorescent was 1.13% / etmal, then the average The third highest average relative growth rate was Bacillus sp 1.12% / etmal and the lowest was 0.69% / etmal in the control treatment.

2. Discussion

Based on the results of seed biopriming treatment using rhizobacteria that can increase the vigor index of cocoa, the results of this study are in line with the research of Sutariati et al. (2014) that the biopriming treatment of Bacillus sp. CKD061 had a better effect on the viability and vigor of cocoa seeds. Seed biopriming treatment increased seed viability and vigor in plants (Moradi and Younesi, 2009; Wahid et al., 2008; Sutariati, 2006; Ilyas et al., 2002). Seed biopriming treatment was carried out to overcome low productivity caused by low seed use. Seed invigoration techniques have been proven to increase seed viability and vigor (Ilyas et al., 2002).

The use of rhizobacteria Bacillus sp CKD061 and Pseudomonas fluorescent PG01, which was integrated with the seed invigoration technique using matrix conditioning red brick powder or husk charcoal powder, was more effective in increasing the viability and vigor of local upland rice seeds (Sutariati et al., 2014).

Besides dissolving phosphate and fixing nitrogen, Bacillus sp CKD061 can produce the growth hormone IAA with a concentration of 346.79 ppm (Sutariati et al. 2010). Bacillus sp CKD061 is also capable of producing gibberellins and cytokinins. (Sheela & Usharani, 2013; Yasmin et al 2009; Timmusk et al., 2005). Bacillus sp CKD061 can also accelerate the decomposition process of organic matter and provide nutrients for plants (Sutariati et al., 2010). IAA hormone is the active form of the auxin hormone found in plants and plays a role in increasing cell development, spurring growth and increasing enzyme activity.

*Pseudomonas fluorescent*able to suppress tungro attacks on rice (Salamiah, 2015) and can suppress downy mildew attacks 20% to 40% on corn plants (Zaenudin et al., 2015). In addition, Pseudomonas fluorescent bacteria were able to produce IAA hormone with a concentration of 4,0178 ppm.

Based on the observations of the use of bio-priming Bacillus sp CKD061, Pseudomonas fluorescent PG01 and a combination of Bacillus sp. with Pseudomonas fluorescent PG01 incresed germination compared to the control (without treatment).

The vigor index of cocoa seeds using rhizobacteria Bacillus sp CKD061, Pseudomonas fluorescent PG01 and the combination of Bacillus sp CKD061 with Psudomonas fluorescent PG01 is in line with research by Sutariati et al. (2014) that Bacillus sp CKD061 treatment gave a good response to cocoa growth.

At the highest relative growth speed, Pseudomonas fluorescent was able to disseminate phosphorus in the soil and increase the availability of nutrients for plants (Sundara et al., 2002).

E. Conclusion

Based on the study results, biopriming treatment using rhizobacteria had the best effect on cocoa seeds' viability, vigor, and growth compared to controls.

Acknowledgment

Thank you to the research team and those who have contributed to this research.

F. References

Ashrafuzzaman, M., et al. (2009). Efficiency of Plant Growth Promoting Rhizobacteria (PGPR) for the Enhancement of Rice Growth. African Journal of Biotechnology. 8 (7): 1247-1252.

Directorate General of Plantation General of Plantation, Ministry of Agriculture. (2012). Technical Guidelines for Cocoa Plantation Development. Ministry of Agriculture. Jakarta.

Directorate of Spice & Refreshment Plants, (2009). Cocoa Superior Planting Materials. Directorate General of Plantation. Ministry of Plantations. Jakarta.

El Sora, et. al. (2007). Tryptohan Dependent Production of Indole – 3 – Acetic Acid (IAA) Affects Level of Plant Growth Promotion by Bacillus amyloliquefacient F2B42. American Phytopathological Society. 20(6) : 619-626.

El-Hamshary & Khattab A. (2008). Evaluation of Antimicrobial Activity of Bacillus subtilis and Bacillus cereus and Their Fusant Against Fusarium solani. Research Journal of Cell and Molecular Biology. 2(2),24-29.

Hatta, Muhammad. (2009). Effect of Antioxidant Compounds.

Ilyas S. et al. (2002). Matriconditioning Improved Quality and Protein Level of Medium Vigor Hot Paper Seed. Seed Technol. 24 : 65-75.

Kapli H. et al. (2017). Effect of Growth Stimulating Rhizobacteria and Drought Tolerance and Soil Microbial Abundance and Activity on Corn (Zea mays L.). Biospecies Vol. 10(1): 25-36.

Maemunah & Adelina E. (2009). Storage Duration and Invigoration of Vigor of Cocoa Seeds (*Theobroma cacao* L.). Central Sulawesi Research and Development Media 2009 Oct 2(1) : 56-61. ISSN : 1979 – 5971.

Moradi, A., & Younesi, O. (2009). Effects of Osmo-And Hydro-Priming on Seed Parameters of Grain Sorghum (*Sorghum Bocolor* L). Australian Journal of Basic and Applied Sciences, 3(3):1696-1700.

Indonesian Coffee & Cocoa Research Center. (2010). Cocoa Cultivation. Agromedia Library. Jakarta.

Sheela, T. & Usharani. (2013). Influence of plant growth promoting rhizobacteria (PGPR) on the growth of maize (Zea mays L.) Golden research Thoughts.

Siregar, Tumpal HS, Riyadi, Slamet, & Nuraeni L. (2008). Chocolate. Self-help spreader. Jakarta.

Sundara, et al. (2002). Influence of Phosphorous Solubilizing Bacteria on the changes in soil available Phosphorous and Sugarcane and Sugar Yields. Field Crop Res. 77:43-49. S0378-4290(02)00048-5.

Susanto, FX. (1997). Cocoa Plants. Canisius. Jakarta.

Sutariati GAK, Safuan LO, Khaeruni A., & Handayani F. (2014). Effectiveness Test of Biopriming Techniques and Seed Sources on Viability and Vigor of Cocoa Seeds. J. Agriplus, Vol. 24. No: 02 May : 0854-0128.

Sutariati GAK, Safuan LO., & Wahab A. (2012). Physiological Characteristics and Performance of Southeast Sulawesi Indiginous Rhizobacteria as a Growth Stimulator of Chili Plants. J. Hort. 22(1):57-64.

Sutariati GAK, Zul'aiza, Darsan S, Kasra LD.MA, Wangadi S, & La Mudi. (2014). Invigorating Local Gogoh Rice Seeds to Increase Vigor and Overcome Postharvest Physiological Dormancy Problems. *Journal of Agrotechnos*. *4*(1): 10-17.

Sutariati, GAK. (2009). Conditioning Seeds with Rhizobacteria to Improve Physiological and Pathological Quality of Pre-planted Chili Seeds. Warta-Wiptek *17*(1):7-16.

Sutariati, GAK, & Khaeruni, A., (2009). Integration of Seed Invigoration Techniques Plus Biological Agents to Increase Yield and Seed Quality of Soybean Plants. Proceedings of the National Seminar "Innovation for Farmers and Improving the Competitiveness of Agricultural Products" in collaboration with the East Java Agricultural Technology Study Center with FEATI and the East Java Provincial Agriculture Service.

Sutariati, GAK & Wahab. A. (2011). Isolation and Ability Test of Indigenous Rhizobacteria as Disease Control Agents in Chili Plants. *Journal of Horticulture, 20* (1) :86-95.

Tilak et al. (2005). Diversity of Plant Growth and Soil Healt Supporting Bacteria. Curr. science. *89*(1) :136-150.

Timmusk, S., N et al. (2005). Paenibacillus polymyxa Invades Plant Roots and Forms Biofilms. Environ Apps. Microbiol.71:7292-7300.

Vilane F., et al. (2008). A Novel Role for Trichoderma Secondary Metabolites in the Interactions with Plants. Physiol Mol Plant Pathol 72, 80-86.

Wahid et al. (2008). Priming- Induced Metabolic Changes in Sunflowers (*Helianthus annus*) Achenes Improve Germination and Seedling Growth. Botanical Studies 49 : 343-350.

Yasmin et al. (2009). Characterization of beneficial properties of plant growth-promoting rhizobacteria isolated from sweet potato rhizosphere. Africa Journal of Microbiological Research.

Zaenudin, et al. (2014). Effect of PGPR (*Bacillus subtilis* and *Pseudomonas fluorescent*) on Downy mildew in Corn (*Zea mays* L).