



## Effectiveness of Bio-Invigoration Technique against Viability and Vigor of Some Cocoa Seed Source

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### Abstract

The research was aimed to evaluate the effectiveness of bio-invigoration treatment and seed sources on seed viability and vigor. The experiment was conducted in Agronomy Laboratory Unit of Agriculture Faculty of Haluoleo University, from January to May 2013. Laboratory research was arranged in split plot completely design. The main factor was variety which consisted of 3 varieties i.e. Hybrid (V1), Sulawesi 1 (V2) and Sulawesi 2 (V3). The sub plot was seed bio-osmoconditioning with rhizobacteria treatments which consisted of 6 treatments, namely: without seed bio-osmoconditioning (B0), seed bio-osmoconditioning with *Bacillus* sp. CKD061 (B1), seed bio-osmoconditioning with *P. fluorescens* PG01 (B2), seed bio-osmoconditioning with *S. liquefaciens* SG01 (B3), seed bio-osmoconditioning with *Trichoderma* sp. (B4), and seed osmoconditioning with  $KNO_3$  (B5). Every treatment was replicated 3 times. Therefore, overall there were 54 experimental units. Data obtained were analyzed using analysis of variance and followed with Duncan's Multiple Range Test. The result of experiment in the Laboratory showed that *Bacillus* sp. CKD061 and *Trichoderma* sp. were effective in improving viability and vigor of all seed sources of cocoa seed used. In all seed sources used (Hybrid, Sulawesi 1, and Sulawesi 2), these treatment were effective in increasing germination power, homogenous growth, index vigor, and growth compared with untreated treatment. For the best result, still needed further research to evaluated stability effect of seed bio-osmoconditioning on cocoa seedling in the field.

**Keywords:** bio-invigoration, bio-osmoconditioning, rhizobacteria, cocoa seedling .

## A. Introduction

Cocoa (*Theobroma cacao* L.) is one of the national commodity and plays an important role for the Indonesian economy, especially in terms of farmers' income, especially as a provider of jobs and a source of foreign exchange. Cocoa production is currently 435,000 tons to the production of smallholder plantations around 87%. The highest production of 67% was obtained from the area of cocoa production center centered in South Sulawesi, Southeast Sulawesi and Central Sulawesi. Early expansion of cocoa was performed about 25 years ago. This means that the cocoa plantations in Indonesia have been quite old that result in poor productively. Results showed that cocoa plants have aged 25 years of productivity lived half of its production potential (Suhendy, 2007). The low productivity of cocoa in general due not to use the clones/varieties and plants mostly aged older/broken as well as the low level of maintenance done by the planters.

Some factors contributing to low productivity and cocoa quality Indonesia today is the plant material is varied and unclear source, age of the plants that are old, cultivation technical implementation is very low which contributes to the high levels of pests and plant diseases. Efforts are being made to increase the productivity of cocoa is the rejuvenation and rehabilitation of the plants with the use of superior planting materials among hybrid varieties, clones Sulawesi 1, and clones Sulawesi 2. Hybrid varieties are varieties of the cross between Na 32 and UIT 1. Hybrid varieties have high yield, good quality (large seeds), and resistant to pests and diseases. Clones Sulawesi 1 and Sulawesi 2 are cocoa Lindak with purple beans, which is also resistant to pests and diseases, as well as high productivity.

One effort to improve cocoa seed vigor is through bio-engineering invigorasi seed. Bio-invigoration seed Technique can be done by matricconditioning seed or seed osmoconditioning (Ilyas, 2005). Matricconditioning is conditioning by using the media moist solids that have high water holding power. Osmoconditioning seed is conditioning by using osmotic solution such as KNO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub>, NaCl and matinol that is not toxic to the seeds. One treatment osmoconditioning effective and relatively inexpensive is by using osmotic solution in the form of a salt KNO<sub>3</sub>. KNO<sub>3</sub> solution of one of its functions is to accelerate the acceptance of oxygen by the seed.

This study aimed to evaluate the effectiveness of treatment of bio-invigoration and seed source in increasing its viability and vigor as well as information and reference technologies for further research as well as the technology used to be one of the methods that are effective, efficient and environmentally friendly to increase the viability and vigor cocoa seed.

## B. Methodology

The study design used was completely randomized design (CRD) in a split plot pattern. The main plot is the seed source consists of three levels, namely: Hybrid (S0), Sulawesi 1 (S1), and Sulawesi 2 (S2). The subplots were perlakuan bio-invigoration (bio-osmoconditioning) seeds, which consists of six treatments, namely: control (B0), bio-osmoconditioning *Bacillus* sp. CKD061 (B1), bio-osmoconditioning *P. fluorescens* PG01 (B2), bio-osmoconditioning *S. liquefaciens* SG01 (B3), bio-osmoconditioning *Trichoderma* sp. (B4), and osmoconditioning KNO<sub>3</sub> (B5). Each treatment was repeated 3 times, so overall there are 54 experimental units.

The medium used for the multiplication of bacteria that TSA and TSA King's B media made of a mixture to be 20 g and 30 g TSB. Whereas for the manufacture of King's B media consists of a mixture to be 20 g, protease peptone 20 g, glycerol 15 ml, 2.5 g K<sub>2</sub>HPO<sub>4</sub>, and MgSO<sub>4</sub>·7H<sub>2</sub>O 6 g. The mixture of materials for the manufacture of TSA media and King's B were dissolved in 1000 ml of distilled water and boiled for ± 20 minutes. A mixture of materials that have been incorporated into the boiling flask and sterilized using an autoclave (T 121o C, p 1 atm, t 20 minutes). After that, the mixture is poured into a petri dish 0.5 cm thick aseptically in a laminar air flow cabinet then cooled and ready for use. Before it is used, rizobakteri grown in advance in solid TSA medium (for *Bacillus* sp. CKD061 and *S. liquefaciens* SG01), King's B (for *P. fluorescens* PG01), and PDA (for *Trichoderma* sp.) and incubated for 48 hours. Bacterial colonies growing suspended in sterile distilled water to a population density of 10<sup>9</sup> cfu / mL (one loop OSE in 10 ml of sterile distilled water). Seeds soaked in KNO<sub>3</sub> and also suspension of *Bacillus* sp. CKD061, suspension isolates of *P. fluorescens* PG01, isolates of *S. liquefaciens* SG01 suspension, and the suspension of *Trichoderma* sp. for 8 hours. Seeds were planted in a box of 20 grain germination of seeds for each treatment. Maintenance is carried out during the execution of the research that is watering two times a day (morning and afternoon) and adapted to the growing medium moist. Observations were made on the viability and vigor. Observations on viability of seeds were germination (DB). Observation of the vigor which grows simultaneity (KST), vigor index (IV), and relative growth rate (KCT-R).

## C. Result

### 1. Germination

Treatment of bio-osmoconditioning seed better able to increase germination hybrid cocoa seeds, Sulawesi 1, and Sulawesi 2 compared to control (Figure 1). Compared with control, seed treatment using bio-osmoconditioning better able to increase seed germination hybrid cocoa, Sulawesi 1, and Sulawesi 2. among the six kinds of treatment of bio-osmoconditioning used, bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. give germination percentage higher than the bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *S. liquefaciens* SG01, and osmoconditioning KNO<sub>3</sub> on hybrid seed sources, Sulawesi 1, and Sulawesi 2. The hybrid cocoa seeds germination, Sulawesi 1, and Sulawesi 2 lowest was at the control and significantly different from other treatments (Figure 1). Meanwhile, three sources of seeds used, cocoa seed germination Hybrid performing better than the cacao seed Sulawesi Sulawesi 1 and 2 (Table 1).

**Table 1. Effect of bio-osmoconditioning seed treatment and seed source on germination of seeds of cacao hybrids, Sulawesi 1, and Sulawesi 2**

Seed	Source Seed			Mean <i>Bio-Osmo</i>
	Hybrid	Sulawesi 1	Sulawesi 2	
	Germination (%)			
Control	68.33 c P	41.67 c Q	41.67 d Q	50.56
<i>Bio-Osmo</i> CKD061	96.67 a P	95.00 a P	95.00 a P	95.56
<i>Osmo Bio-PG01</i>	95.00 a P	88.33 ab Q	86.67 bc Q	90.00
<i>Osmo Bio-SG01</i>	93.33 a P	86.67 b P	86.67 bc P	88.89
<i>Bio-Osmo Tricho</i>	96.67 a P	95.00 a P	93,33 ab P	95.00
<i>Osmo</i> KNO <sub>3</sub>	81.67 b P	85.00 b P	80.00 c P	82.22
Mean varieties	88.61	81.95	80.56	

Description: The numbers followed by the same lowercase letters in the same column, and figures followed the same capital letters on the same line showed no significant effect on the level of DMRT 0.05.

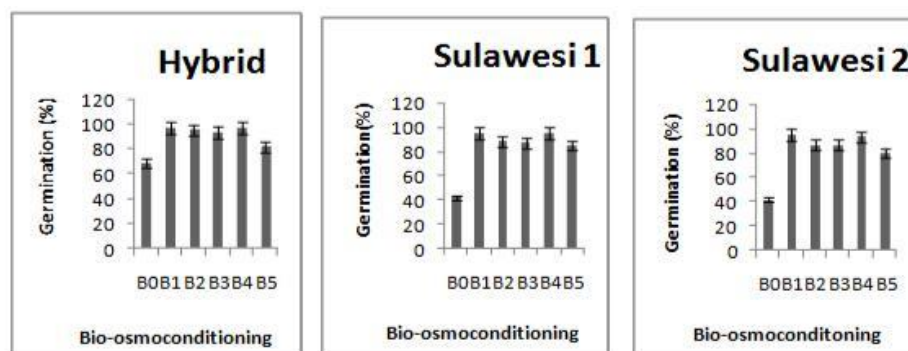


Figure 1. The hybrid cocoa seeds germination, Sulawesi 1 and 2 are chances, Sulawesi treatment of bio-osmoconditioning. B0 (control), B1 (bio-osmoconditioning *Bacillus* sp. CKD061), B2 (bio-osmoconditioning *P. fluorescens* PG01), B3 (bio-osmoconditioning *S. liquefaciens* SG01), B4 (bio-osmoconditioning *Trichoderma* sp.), B5 ( osmoconditioning KNO<sub>3</sub>).

### 2. Growing simultaneity

Treatment of bio-osmoconditioning seeds are also better able to increase simultaneity grow cacao seeds Hybrids, Sulawesi 1, and Sulawesi 2 compared with controls. Among the six bio-osmoconditioning treatment is used, the hybrid cocoa seeds bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. provide simultaneity percentage grows higher as compared with the treatment of bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *S. liquefaciens* SG01, and osmoconditioning KNO<sub>3</sub>. On Sulawesi cocoa seeds 1, only the treatment of bio-osmoconditioning *Bacillus* sp. CKD061memberikan simultaneity percentage grows higher than the bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *S. liquefaciens* SG01, bio-osmoconditioning *Trichoderma* sp., and osmoconditioning KNO<sub>3</sub>. While at the Sulawesi cocoa seeds 2, which gives the percentage grows higher simultaneity is bio-osmoconditioning *Bacillus* sp. CKD061, bio-osmoconditioning P.

fluorescens PG01, and bio-osmoconditioning *Trichoderma* sp. Simultaneity growing hybrid cocoa seeds, Sulawesi 1, and Sulawesi 2, lowest was at the control and significantly different from other treatments (Figure 2). Meanwhile, three sources of seeds used, hybrid cocoa seeds demonstrate the performance simultaneity grows better than the cacao seed Sulawesi Sulawesi 1 and 2 (Table 2).

**Table 2. Effect of treatment of bio-osmoconditioning seed and seed source of the simultaneity grow cacao seeds Hybrids, Sulawesi 1, and Sulawesi 2**

Bio-osmoconditioning Seed	Source Seed			Mean <i>Bio-Osmo</i>
	Hybrid	Sulawesi 1	Sulawesi 2	
Growing simultaneity (%)				
Control	58.33 c P	36.67 d Q	36.67 b Q	50.56
<i>Bio-Osmo</i> CKD061	90.00 a P	86.67 a P	63.33 a Q	95.56
<i>Osmo Bio-PG01</i>	73.33 b P	68.33 bc P	60.00 a P	90.00
<i>Osmo Bio-SG01</i>	78.33 ab P	68.33 bc P	45.00 bc Q	88.89
<i>Bio-Osmo Tricho</i>	90.00 a P	75.00 b PQ	61.67 a Q	95.00
<i>Osmo</i> KNO <sub>3</sub>	75,00 ab P	63.33 c PQ	56.67 ab Q	82.22
Mean varieties	77,50	66.39	53.89	

Description: The numbers followed by the same lowercase letters in the same column, and figures followed the same capital letters on the same line showed no significant effect on the level of 0.05 DMRT.

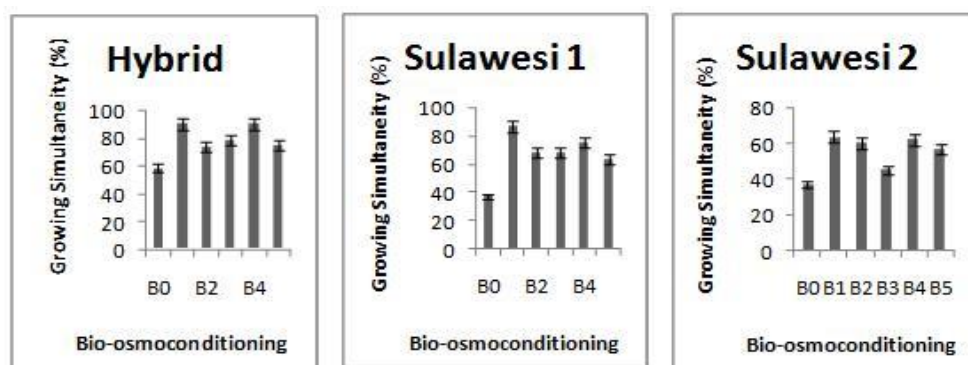


Figure 2. Simultaneity growing hybrid cocoa seeds, Sulawesi 1, and Sulawesi 2 were treated bio-osmoconditioning. B0 (control), B1 (bio-osmoconditioning *Bacillus* sp. CKD061), B2 (bio-osmoconditioning *P. fluorescens* PG01), B3 (bio-osmoconditioning *S. liquefaciens* SG01), B4 (bio-osmoconditioning *Trichoderma* sp.), B5 (osmoconditioning KNO<sub>3</sub>).

### 3. Index of Vigor

Among the six bio-osmoconditioning treatment that are used, the hybrid cocoa seeds bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. give vigor index percentage is higher than the bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *S. liquefaciens* SG01, and osmoconditioning KNO<sub>3</sub>. At first Sulawesi cocoa seeds, bio-osmoconditioning *Bacillus* sp. CKD061 give vigor index percentage is higher than the bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *S. liquefaciens* SG01, bio-osmoconditioning *Trichoderma* sp., and osmoconditioning KNO<sub>3</sub>. While at the Sulawesi cocoa seeds 2, which gives a high percentage vigor index is bio-osmoconditioning *Trichoderma* sp., and osmoconditioning KNO<sub>3</sub>. The lowest Hybrid cocoa seed vigor index was at the control and significantly different from other treatments. In Sulawesi 1 seed, the lowest vigor index was at the control and significantly different from the treatment of bio-osmoconditioning *Bacillus* sp. CKD061 but no significant treatment with bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *S. liquefaciens* SG01, bio-osmoconditioning *Trichoderma* sp., and osmoconditioning KNO<sub>3</sub>. While at the Sulawesi 2cocoa seeds, the lowest vigor index was at the control and significantly different from the treatment of bio-osmoconditioning *Bacillus* sp. CKD061 bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *Trichoderma* sp., and osmoconditioning KNO<sub>3</sub> but no significant effect on the treatment of bio-osmoconditioning *S. liquefaciens* SG01 (Figure 3).

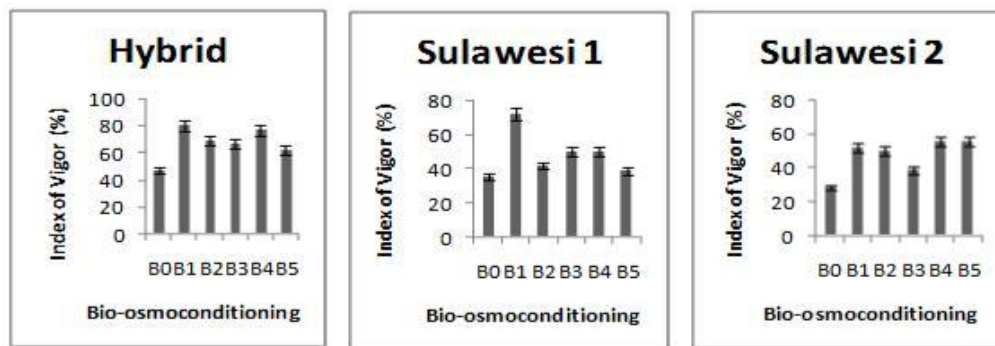


Figure 3. Hybrid cocoa seed vigor index, Sulawesi 1, and Sulawesi 2 were treated bio-osmoconditioning. B0 (control), B1 (bio-osmoconditioning *Bacillus* sp. CKD061), B2 (bio-osmoconditioning *P. fluorescens* PG01), B3 (bio-osmoconditioning *S. liquefaciens* SG01), B4 (bio-osmoconditioning *Trichoderma* sp.), B5 ( osmoconditioning KNO<sub>3</sub>).

**Table 3. Effect of bio-osmoconditioning seed treatment and seed source of the cacao seed vigor index Hybrids, Sulawesi 1, and Sulawesi 2**

Bio-osmoconditioning	Source Seed			Mean
	Hybrid	Sulawesi 1	Sulawesi 2	
Seed	Vigor Index (%)			Bio-Osmo
Control	46.67 c P	35.00 b PQ	28.33 c Q	36.67
Bio-Osmo CKD061	80.00 a P	71.67 a P	51.67 a Q	67.78
Osmo Bio-PG01	68.33 ab P	41.67 b Q	50.00 ab Q	53.33
Osmo Bio-SG01	66,67 ab P	50.00 b Q	38.33 bc Q	51.67
Bio-Osmo Tricho	76.67 ab P	50.00 b Q	55.00 a PQ	60.56
Osmo KNO <sub>3</sub>	61.67 b P	38.33 b Q	55.00 a P	51.67
Mean varieties	66.67	47.78	46.39	

Description: The numbers followed by the same lowercase letters in the same column, and figures followed the same capital letters on the same line showed no significant effect on the level of 0.05 DMRT.

Meanwhile, three sources of seeds used, cocoa seed vigor index Hybrids performed better than the cacao seed Sulawesi 1 and Sulawesi 2 (Table 3).

#### 4. Relative Growing Speed

**Table 4. Effect of bio-osmoconditioning seed treatment and seed source to grow relative speed hybrid cocoa seeds, Sulawesi 1, and Sulawesi 2**

Bio-osmoconditioning	source Seed			Mean
	Hybrid	Sulawesi 1	Sulawesi 2	
Seed	Speed Relative Growth (% / etmal)			Bio-Osmo
Control	74.28 c P	44.67 d Q	43.79 d Q	54.24
Bio-Osmo CKD061	112.61 a P	105.46 a PQ	99.35 a Q	105.81
Osmo Bio-PG01	102.61 a P	Bc 90.67 Q	92,66a b Q	95.31
Osmo Bio-SG01	103.72 a P	91.47 bc PQ	87,84b c Q	94.34
Bio-Osmo Tricho	111.45 a P	Ab 99.90 Q	95.17 a Q	102.17
Osmo KNO <sub>3</sub>	89.94 b P	87.23 c P	85.66 c P	87.61
Mean varieties	99.10	86.57	84.08	

Description: The numbers followed by the same lowercase letters in the same column, and figures followed the same capital letters on the same line showed no significant effect on the level of 0.05 DMRT.

Seed treatment using bio-osmoconditioning able to increase the speed of growth relative hybrid cocoa seeds, Sulawesi 1, and Sulawesi 2 compared to control (Figure 4). Treatment of bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. provide the best relative growth rate compared to the control and treatment of bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *S. liquefaciens* SG01, osmoconditioning KNO<sub>3</sub> in hybrid cocoa seeds. On Sulawesi cocoa seeds 1, only the treatment of bio-osmoconditioning *Bacillus* sp. CKD061 which gives the highest relative growth rate compared with other treatments. While in the Sulawesi 2 cocoa seed, which gives the highest relative percentage growth rate is the

treatment of bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. Relative growth rate was lowest for the control seeds and significantly different from other treatments (Figure 4). Meanwhile, three sources of seeds used, cocoa seed Hybrids show performance relative growth rate is better than the cacao seed Sulawesi 1 and Sulawesi 2 (Table 4).

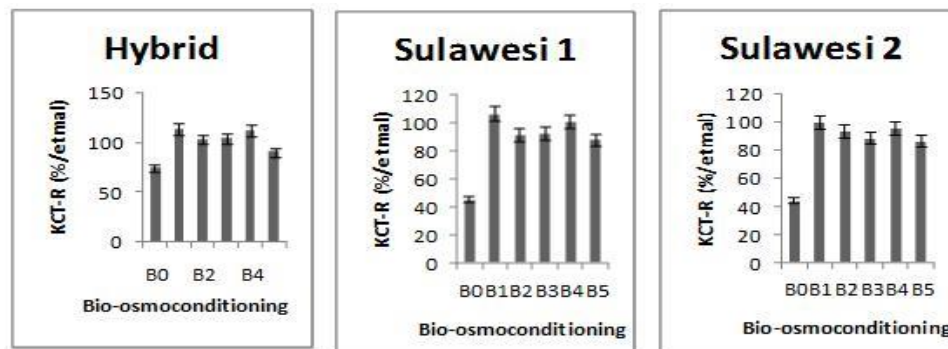


Figure 5. Relative Growth rate of hybrid cocoa seeds, Sulawesi 1, and Sulawesi 2 were treated bio-osmoconditioning. B0 (control), B1 (bio-osmoconditioning *Bacillus* sp. CKD061), B2 (bio-osmoconditioning *P. fluorescens* PG01), B3 (bio-osmoconditioning *S. liquefaciens* SG01), B4 (bio-osmoconditioning *Trichoderma* sp.), B5 (osmoconditioning KNO3).

#### D. Discussion

The results showed that the treatment of bio-invigoration (bio-osmoconditioning) seeds significantly able to improve the viability and vigor of cocoa compared with controls. The results of this study are consistent with results of previous studies showing that the use of bio-invigoration (bio-osmoconditioning) as a seed treatment able to improve the viability and vigor of plants. Bio-osmoconditioning seed treatment proven effective in improving the viability and vigor of plants (Ilyas *et al.*, 2002; Sutariati, 2006; Wahid *et al.*, 2008; Moradi and Younesi, 2009).

Bio-osmoconditioning seed treatment was done to address the low productivity due to the use of seeds bervigor low. Seed vigor was reflected by two informations about viability, respectively the growing strength and storability of seed. Treatment of bio-osmoconditioning seed showed the very real differences between seed treated with seed bio-osmoconditioning the untreated bio-osmoconditioning. The observation of several variables viability and vigor showed that rizobakteri *Bacillus* sp. CKD061 and *Trichoderma* sp. respond more to the cocoa seeds, while *P. fluorescens* PG01, *S. liquefaciens* SG01, and KNO3 not provide a response to the cacao seeds used. Rizo-bacterial colonization by the host plant, begins when the seeds germinate. At the same time, rizobakteri also need adequate nutrition for growth and development. Generally rizobakteri nutrients derived from organic acids released its host, and type of organic acids is certainly different between plants with one another. Therefore, the lack of contributions made by the host rizo-bacterial likely caused by a lack of acquisition of nutrients from its host.

The results showed that the treatment of bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. able to increase the percentage of seed germination compared to the control and treatment of bio-osmoconditioning *P. fluorescens* PG01, bio-osmoconditioning *S. liquefaciens* SG01, osmoconditioning KNO 3 (on a hybrid cocoa seeds, Sulawesi 1, and Sulawesi 2).

The observation of the highest percentage of simultaneity grow hybrid cacao seeds and Sulawesi 2 is shown by the treatment of bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. meanwhile, on Sulawesi cocoa seeds 1, the highest percentage growth synchrony obtained in the treatment of bio-osmoconditioning *Bacillus* sp. CKD061.

Hybrid seed vigor index highest cocoa obtained in the treatment of bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. Meanwhile, on Sulawesi cocoa seeds 1, only the treatment of bio-osmoconditioning *Bacillus* sp. CKD061 are better able to increase the percentage of cocoa seed vigor index. On Sulawesi cocoa seeds 2, which gives the highest percentage vigor index is the treatment of bio-osmoconditioning *Trichoderma* sp. and osmoconditioning KNO3. In observation of relative growth speed hybrid cocoa seeds, Sulawesi 1, and Sulawesi 2, the highest relative growth rate obtained in the treatment of bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. From the

experimental results show that the hybrid seed source provides better performance than the seed source Sulawesi 1 and Sulawesi 2.

Treatment of bio-osmoconditioning *Bacillus* sp. CKD061 in continuity give a better response compared to other treatments and control on plant growth. This is presumably because *Bacillus* sp. CKD061 can produce growth hormone IAA, gibberellins, and cytokinins, in addition to its ability to dissolve phosphate and nitrogen fixation (Timmusk *et al.*, 2005). According to Sutariati & Wahab (2010), *Bacillus* sp. CKD061 capable of producing IAA with a concentration of 346.97 ppm. IAA is the active form of the hormone auxin found in plants and plays in improving cell development, spur growth, and increase the activity of enzymes.

In addition to acting spur plant growth, *Bacillus* sp. also able to increase the availability of nutrients with its ability to fix N & P. Use of Rizo- bacteria dissolving solvent phosphate that can substitute some or the entire crop needs to be an element of P can give positive results on plant growth. Nutrient phosphate is indispensable in the metabolism of plants, among others to stimulate plant growth, root development, fruit growth, improve quality and strengthen resistance to pests and diseases. Dissolution of phosphate is caused by bacteria that produce enzymes that can break phosphate phosphatase bound by organic compounds, for example phospholipids and glycolipids that provides nutrients into a form that is available so that the availability of adequate plant P (Joner *et al.*, 2000). While the ability to embed P of compound P are bound by gibsid due rizobakteri ability to produce organic acids.

The ability of *Trichoderma* sp. spur the growth of the plant has been reported by Hajieghrari (2010) on corn seeds by *Trichoderma*. Isolates of *Trichoderma* sp. is able to increase the length of roots and shoots of corn seeds and to improve the conductivity of stomata. This is because some isolates of *Trichoderma* sp. is able to produce factors that can encourage the growth of plants or produce phytohormones such as Indole Acetic Acid (IAA) and similar hormones (Vinale *et al.*, 2008). In addition, *Trichoderma* sp. is able to accelerate the process of decomposition of organic matter and then provide nutrients for plants (Chet, 2001).

## E. Conclusion

1. All three source of seeds were used in this study (Hybrids, Sulawesi 1, and Sulawesi 2) responsive to the treatment of bio-invigoration (bio-osmoconditioning) seeds were given, indicated by an increase in the viability and vigor of cocoa.
2. Treatment of bio-osmoconditioning *Bacillus* sp. CKD061 and bio-osmoconditioning *Trichoderma* sp. gives a better effect on the viability and vigor of cocoa.

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