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

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 \textit{Bacillus} sp. CKD061 (B1), seed bio-osmoconditioning with \textit{P. fluorescens} PG01 (B2), seed bio-osmoconditioning with \textit{S. liquefaciens} SG01 (B3), seed bio-osmoconditioning with \textit{Trichoderma} sp. (B4), and seed osmoconditioning with KNO3 (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 \textit{Bacillus} sp. CKD061 and \textit{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 Lindar 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 invigoration seed. Bio-invigoration seed Technique can be done by matriconditioning seed or seed osmoconditioning (Ilyas, 2005). Matriconditioning is conditioning by using the media moist solids that have high water holding power. Osmoconditioning seed is conditioning by using osmotic solution such as KNO3, KH2PO4, 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 KNO3. KNO3 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 KNO3 (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 K2HP04, and MgSO4.7H2O 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 109 cfu / mL (one loop OSE in 10 ml of sterile distilled water). Seeds soaked in KNO3 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 KNO3 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 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

| Bio-osmoconditioning | Source Seed | Mean Germination (%) | Bio-Osmo |
|----------------------|-------------|----------------------|---------|
|                      | Hybrid      | Sulawesi 1 | Sulawesi 2 |     |
| Control              | 68.33 c P   | 41.67 c Q  | 41.67 d Q  | 50.56 |
| Bio-Osmo CKD061      | 96.67 a P   | 95.00 a P   | 95.00 a P   | 95.56 |
| Osmo Bio-PG01        | 95.00 a P   | 88.33 ab Q  | 86.67 bc Q  | 90.00 |
| Osmo Bio-SG01        | 93.33 a P   | 86.67 b P   | 86.67 bc P   | 88.89 |
| Bio-Osmo Tricho      | 96.67 a P   | 95.00 a P   | 93.33 ab P   | 95.00 |
| Osmo KNO3            | 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 KNO3).

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 KNO3. On Sulawesi cocoa seeds 1, only the treatment of bio-osmoconditioning Bacillus sp. CKD061 memberikan simultaneity percentage grows higher than the bio-osmoconditioning P. fluorescens PG01, bio-osmoconditioning S. liquefaciens SG01, bio-osmoconditioning Trichoderma sp., and osmoconditioning KNO3. 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  | Source Seed | Mean          |
|-----------------------|-------------|---------------|
|                       | Hybrid      | Sulawesi 1    | Sulawesi 2    |
| Control               | 58.33 c P   | 36.67 d Q     | 36.67 b Q     | 50.56 |
| Bio-Osmo CKD061       | 90.00 a P   | 86.67 a P     | 63.33 a Q     | 95.56 |
| Osmo Bio-PG01         | 73.33 b P   | 68.33 bc P    | 60.00 a P     | 90.00 |
| Osmo Bio-SG01         | 78.33 ab P  | 68.33 bc P    | 45.00 bc Q    | 88.89 |
| Bio-Osmo Tricho       | 90.00 a P   | 75.00 b PQ    | 61.67 a Q     | 95.00 |
| Osmo KNO3             | 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 KNO3).

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 KNO3. 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 KNO3. While at the Sulawesi cocoa seeds 2, which gives a high percentage vigor index is bio-osmoconditioning Trichoderma sp., and osmoconditioning KNO3. 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 KNO3. While at the Sulawesi 2 cocoa 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 KNO3 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 KNO3).

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 |
| Control              | 46.67 c P   | 35.00 b PQ | 28.33 c Q  |
| Bio-Osmo CKD061      | 80.00 a P   | 71.67 a P | 51.67 a Q  |
| Osmo Bio-PG01        | 68.33 ab P  | 41.67 b Q | 50.00 ab Q |
| Osmo Bio-SG01        | 66.67 ab P  | 50.00 b Q | 38.33 bc Q |
| Bio-Osmo Tricho      | 76.67 ab P  | 50.00 b Q | 55.00 a PQ |
| Osmo KNO3            | 61.67 b P   | 38.33 b Q | 55.00 a P  |
| 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 |
| Speed Relative Growth (% / etmal) |
| Control              | 74.28 c P   | 44.67 d Q | 43.79 d Q  |
| Bio-Osmo CKD061      | 112.61 a P  | 105.46 a PQ | 99.35 a Q  |
| Osmo Bio-PG01        | 102.61 a P  | Bc 90.67 Q | 92.66a b Q |
| Osmo Bio-SG01        | 103.72 a P  | 91.47 bc PQ | 87.84b c Q |
| Bio-Osmo Tricho      | 111.45 a P  | Ab 99.90 Q | 95.17 a Q  |
| Osmo KNO3            | 89.94 b P   | 87.23 c P | 85.66 c P  |
| 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 KNO3 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.

**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 hervigor 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 KNO3 (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.

**F. References**

Chet, I. (2001). Effect of Trichoderma harzianum on Microelement Concentrations and Increased Growth of Cucumber Plants. Plant Soil, 235(2), 235-242.

Hajieghrari, B. (2010). Effects of Some Iranian Trichoderma Isolates on Maize Seed Germination and Seedling Vigor. *African J Biotech*, 9(28), 4342-4347.

Ilyas, S. (2005). Perubahan Fisiologis dan Biokimia dalam Proses Seed Conditioning. *Keluarga Benih*, Vol. VI, 2: 70-79.

Ilyas, S., Sutariati, G.A.K., Suwanto & F.C., Sudarsono. (2002). Matriconditioning Improved Quality and Protein Level of Medium Vigor Hot Pepper Seed. *Seed Technol*, 24:65-75.

Joner, E.J., I.M., Aarle & M., Vosatka. (2000). Phosphatase Activity of Extraradical Arbuscular Mychoriza hyphae: A review. *Plant Soil* 226:190-210.

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

Suhendry. (2007). Rehabilitasi Tanaman Kakao: Tinjauan Potensi, Permasalahan, Rehabilitasi Tanaman Kakao di Desa Primatani Tonggolobibi. Prosiding Seminar Nasional 2007. Pengembangan Inovasi Pertanian Lahan Marginal. Departemen Pertanian.

Sutariati, G.A.K & A., Wahab. (2010). Isolasi dan Uji Kemampuan Rizobakteri Indigenus sebagai Agensia Pengendali Hayati Penyakit pada Tanaman Cabai. J. 20(1): 86-95.

Timmusk, S., N. Grantcharova & E.G.H., Wagner. (2005). Paenibacillus polymyxa Invades Plant Roots and Forms Biofilms. Appl. Environ. *Microbiol*, 71:7292-7300.

Vinale, F., K. Sivasithamparam, E.L. Ghisalberti, R. Marra, M.J., Barbetti, H. Li, S.L., Woo & M. Lorito. (2008). A Novel Role for Trichoderma Secondary Metabolites in the Interactions with Plants. *Physiol Mol Plant Pathol* 72, 80-86.
Wahid, A., Noreen A., Shahzad, M.A., Basra., Gelani., S. & Farooq, M. (2008). Priming-Induced Metabolic Changes in Sunflower (Helianthus annuus) Achenes Improve Germination and Seedling Growth. *Botanical Studies* 49: 343-350.