

## Effectiveness *Trichoderma asperellum* on the Growth Cocoa Seeds under the Old Cocoa Trees

Marliana S. Palad<sup>1</sup>, Ambo Ala<sup>2</sup>, Nasaruddin<sup>2</sup> & Nadira Sennang<sup>2</sup>

<sup>1</sup>Program of Agricultural Sciences, Graduate Hasanuddin University, Indonesia

<sup>2</sup>Agroteknologi Studies Program, Faculty of Agriculture, Hasanuddin University, Indonesia

Correspondence: Marliana S.Palad, Program of Agricultural Sciences, Graduate Hasanuddin University, Makassar 90245, Indonesia. Tel: 62-8529-980-1395. Email: lallypalad@yahoo.co.id

Received: June 11, 2016

Accepted: July 3, 2016

Online Published: August 17, 2016

doi:10.5539/mas.v10n11p176

URL: <http://dx.doi.org/10.5539/mas.v10n11p176>

### Abstract

This study aimed to determine the effect of *Trichoderma asperellum* on the growth of cocoa seedlings under cocoa stands around 20 years old, so that cocoa seedlings to be used as rootstock on approach grafting for the rehabilitation of the roots of cocoa plants are old, growing well. Research compiled by randomized block design with frequency variation application *T. asperellum* that, once, twice and three times application respectively of 4 gr.L<sup>-1</sup> each plant. The results of the research, granting *T. asperellum* on cocoa seedlings planted under old cocoa trees and is still productive, able to help overcome competition in the utilization of nutrients and water as well as other growth inhibiting factors, and the influential on the vegetative growth of cocoa seedlings. Frequency of the best is three times the application that provides the most effective results for growing cocoa seeds, the seed growing percentage of 100% and an average plant height of 144.45 cm; number of leaves 37 strands; stem diameter 12.22 mm; and total leaf area 1388.30 cm<sup>2</sup>.

**Keywords:** frequency of application, MCC-01, old cocoa, *Trichoderma asperellum*

### 1. Introduction

Cocoa (*Theobroma cacao* L.) is the essential raw commodity for the world's chocolate industry, which was worth \$150bn in 2014. By 2016, the global chocolate market will be worth \$98.3 billion. Indonesian is the world's third leading producer of cocoa beans after Ghana and Ivory Coast, growing 740.500 tonnes in 2012, according to the FAO (Worldatlas, 2015).

West Sulawesi contributed 20% of the national cocoa production, and is the largest cocoa producer in Indonesia, with the area planted in 2014 is 172,000 hectares with a production of 110-115 thousand tons per year (Anonymous, 2014). These data indicate that cocoa is the backbone of the economy in West Sulawesi, but ironically in West Sulawesi cocoa productivity continues to decline. Several factors are significant on the level of productivity of cocoa, besides extensive cocoa plantations, varieties, pests and diseases, climate anomalies, the plant canopy is damaged, as well as age older plants cause less productive plants (Anaeani & Frimpong, 2013). In addition, the dominant limiting factors increasing of cocoa productivity is soil fertility including low organic matter content, low exchange able minerals nutrients such as K and low soil CEC (Ololade, 2010).

Side-grafting is the introduction of cultivation technology that quickly spread among farmers, which initially gives hope bright enough in order to improve the productivity of cocoa. However, after the results are satisfactory side-grafting produce multiple harvests, further crop losses in production and productivity. This is likely caused by an imbalance of the capability of the plant canopy to produce, with the condition that the old plant root system in supplying the needs of nutrients and water from the roots.

The work can be done to fix the root system of the tree cacao side grafting is a technique of approach-grafting, by utilizing the cocoa seeds that have been aged for at least 6 months, so that plant roots can be rehabilitated and is expected cocoa productivity can be improved. The success of this effort is especially if the cocoa seedlings that will be continued to grow well. Cocoa seedlings were planted under old cocoa stand side grafting productive results, will have competition in the use of water and nutrients to the vegetative growth.

One functional microorganism that is widely known as a biological fertilizer the soil is *Trichoderma* sp. These

microorganisms are fungi occupants of the land that can be isolated from plant roots field, including cocoa. Species *Trichoderma* as well as decomposing organisms, can also function as biological agents and plant growth stimulator (Okoth et al., 2011; Anad & Reddy, 2009; Saba et al., 2012). For example *Trichoderma harzianum* give the same response to auxin in improving the cocoa plant roots extension (Nurahmi et al., 2012). *Trichoderma* also produce phytohormones ET and IAA, which plays a role in the sustainability of the growth of plants and plant resistance to disease control and adverse environmental conditions (Hermosa et al., 2012).

Based on this, *Trichoderma* sp. is expected to help overcome the competition to get water and nutrients and factors inhibiting the growth of other, so the cocoa seedlings to be used as rootstock on approach grafting for the rehabilitation of the roots of cocoa plants are old, can grow well.

The results of research on the role of *Trichoderma* sp. of the cocoa plant is a lot, especially as disease control in cocoa, such as leaf blight, pod disease and cancer stem caused by *Phytophthora palmivora* (Asti et al., 2013; Adebola & Amadi, 2012). Bae et al. (2009) reported that *Trichoderma* sp. resulted in cocoa seedlings are more tolerant to drought. Technology of *Trichoderma* sp. is growing rapidly, but the information about the role of *Trichoderma* sp. in addressing competition cocoa seedlings were planted in the area around the plant roots productive yet available. This study aimed to determine the effect of *Trichoderma* sp. to the growth of cocoa seedlings under stands of old cocoa productive.

## 2. Materials and Methods

The experiment was conducted in farm field of Bunde village, Sampaga district, Mamuju regency, West Sulawesi. Six months old cocoa seedlings planted under the cocoa tree stand of around 20 years old, with a spacing of 3 x 3 m. Planting hole was made with size of 40 x 40 x 30 cm 3 hole, and in distance of 20 cm from old cocoa tree.

The materials used are the seeds of *Theobroma cacao* variety Masamba Cocoa Clone (MCC) 01, *Trichoderma asperellum* isolates ART-4 were obtained from Laboratory of Microbiology Faculty of Agriculture University of Hasanuddin, manure, NPK, meter, shove, and so on. The experiments used designed randomized block design (RBD) with frequency treatment applications of *T. asperellum*. The provision of *T. asperellum*: one time, two times, three times application of *T. asperellum* respectively of 4 gr.L<sup>-1</sup> each plant and without application. The first application was given at the time of planting and subsequent intermittent applications a week later. Each treatment was repeated 3 times, so the total plants totaling 36 plants observed.

To assess the effect of treatment, it was carried out observations of parameters including average plant height (cm) measured from a height of 2 cm from ground level to the growing point of the top, the average number of leaves (leaf) formed, average trunk diameter (mm) 5 cm from ground level, and the average total leaf area (cm<sup>2</sup>) was measured using graph paper (Nasaruddin, 2011) with the formula:

$$LD = n \times lk(1)$$

LD is the leaf area (cm<sup>2</sup>), n is the number of boxes, and lk is the area of each box (1 cm<sup>2</sup>)

To determine the effect of treatment on the parameters observed, an analysis of variance and if there is a treatment effect were significantly different followed by Least Significant difference test (LSD) with significance level of 5%.

## 3. Results and Discussion

Observations were done to determine the physical and chemical properties of the soil. The results of the analysis of soil chemical properties in the study area is a total-N, C / N ratio, organic C, K and Na is low, pH, Ca and P<sub>2</sub>O<sub>5</sub> is classified, and Mg and CEC is high and the type of clay clayey (Tab. 1).

Table 1. Chemical and physical properties of soil in the experiment

soil chemical properties	
pH H <sub>2</sub> O	6,2
N total (%)	0,11
organic C (%)	1,08
C / N ratio	10
P <sub>2</sub> O <sub>5</sub> (ppm)	10,9
Ca (cmol / kg)	6,84
Mg (cmol / kg)	3,52
Na (cmol / kg)	0,19

CEC (cmol / kg)	25,66
soil texture	
Sand (%)	32
dust (%)	30
Clay (%)	38

Note. Source: Lab. Chemistry and Soil Fertility, Soil Department Agriculture Faculty, Hasanuddin University

The provision of *T. asperellum* was very influential on the vegetative growth of cocoa seedlings planted under old cocoa stand still in production (Tab. 2).

Table 2. Effect of Frequency Applications *T. asperellum* of the vegetative growth of cocoa seedlings age of 12 weeks after treatment applications in the field

Treatment	Height Plant (cm)	Number of leaves (leaf)	Stem Diameter (mm)	Total leaf area (cm <sup>2</sup> )	Percentage Growth (%)
without application	109.33 a	24.56 a	8,55	999,72 a	52 a
one-time application	134 b	32.89 b	10,89	1357,22 b	83 b
two-times application	134.44 b	32.78 b	10,44	1302,84 b	94 bc
three-times application	144.45 c	37 c	12,22	1388,30 b	100 c
LSD $\alpha$ 0.05	8.90	2.05	tn	130.79	13.21
CV (%)	5.91	5.59	12.35	8.98	15.27

Note. Description: Figures followed by the same letters in the same column are not significantly different according to LSD test level of 5%

Growth of cocoa seedlings the highest and have the most was the number of leaves on the seedlings by *T. asperellum* for three times application and significantly different from other treatments. In total leaf area, giving *Trichoderma asperellum* once, two times and three times did not differ very significantly different result but with no provision of this fungus. Giving *T. asperellum* did not significantly affect seedling stem diameter, but the percentage of seedlings grown greatly affected by the fungus is mainly on the treatment two to three times as many applications. This indicates that the application of *T. asperellum* can increase vegetative growth is better than without the provision of *T. asperellum*.

The foregoing is highly correlated with the intensity of growth (elongation rate) shoots high. As we know that the growth of the plant involves a number of processes, such as multiplication and differentiation of cells (Basri, 2004). Cell multiplication caused more cell mass (as in the elongation and enlargement of the trunk diameter) while the cell differentiation leads to formation of tissues or organs (such as increasing the number of leaves). Thus, it is clear that with the increase of the length and diameter of the rod will be accompanied by the establishment organs, such as the size (length and width) as well as the number of leaves on shoots of cocoa. As we know that there are a number of factors that influence the growth of planting material, including external factors (environment such as climate, soil and applied technology) and internal factors (genetic including the quality and size of the mass meristem cells contained in a planting material) (Fosket, 1999; Hopkins, 1999).

The regression analysis in Figure 1 show that application of *T. asperellum* positively correlated linearly with the vegetative growth of cocoa seedlings age of 12 weeks after treatment. The more the frequency of application of *T. asperellum* increasingly better the vegetative growth of cocoa seedlings.

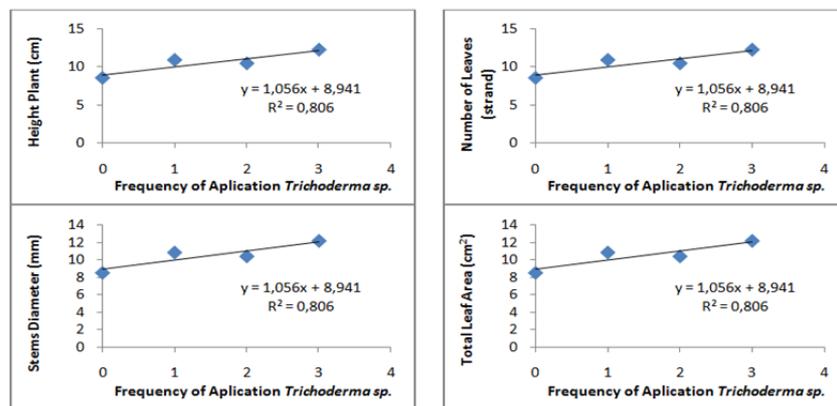


Figure 1. Graph of the regression equation vegetative growth of cocoa seedlings age of 12 weeks after treatment applications in the field

Based on the figure above shows that each increase of one unit of frequency applications *T. asperellum* with a dose of 4 gr.L<sup>-1</sup> will increase by 7.07 cm plant height, leaf number 3.72 strands, stem diameter of 1.06 mm and leaf area of 111.1 cm<sup>2</sup>. Giving *T. asperellum* able to increase vegetative growth of cocoa seedlings, because fixing *rhizosphere* plant roots and helps decompose soil organic matter into nutrients that plants need for growth (Adedeji, 2008). The existence of *T. asperellum* on the ground can improve the physical properties (porosity) and soil fertility. Conditions arable land with good soil aggregation can stimulate vegetative growth of plants.

*Trichoderma asperellum* can multiply rapidly in the *rhizosphere*, and a parasitic fungus that can attack and take nutrients from other fungi (antagonist). The role of *Trichoderma sp.* antagonistic and at the same rapid breeding in the *rhizosphere* make the existence of this fungus can act as a *biocontrol* against fungi that cause diseases (Harman, 2011) for example *Phytophthora palmivora* causes stem rot disease, the fruit and leaves of the cocoa that can improve plant growth

#### 4. Conclusion

Granting *Trichoderma asperellum* on cocoa seedlings planted under old cocoa trees and is still productive, able to help overcome competition in the utilization of nutrients and water as well as other growth inhibiting factors, and the influential on the vegetative growth of cocoa seedlings.

Frequency of *Trichoderma sp.* the best is three times the application that provides the most effective results for growing cacao seeds, the seed growing percentage of 100% and an average plant height of 144.45 cm; number of leaves 37 strands; stem diameter 12.22 mm; and total leaf area 1388.30 cm<sup>2</sup>.

#### Acknowledgments

We would like to thanks the General Directorate of Science-Tecnology Resources and Higher Education in the Ministry of Research, Technology and Higher Education.

#### References

- Adebola, M.O., & Amadi, J.E. (2012). Studies on *Penicillium digitatum*, *Botrydiploidia theobromae*, *Alternaria tenuis* and *Trichoderma harzianum* for Bicontrol of *Phytophthora palmivora*. *Cocoa Black Pod Disease Pathogen. American-Eurasian Journal of Agronomy*, 5(2), 30-34. <http://dx.doi.org/10.5829/idosi.aeja.2012.5.2.2046>
- Adedeji, A. R., Odebode, A. C., & Agbeniyi, S. O. (2008). Bioassay of Five *Trichoderma* Strains Against *Phytophthora megakarya* (Cacao Pod-rot) in Nigeria. *Scientific Research and Essay*, 3(9), 390-394. Retrieved from <http://www.academicjournals.org/SRE>
- Anaeani, F., & Frimpong, O.K. (2013). An Analysis of Yield Gap and Some Factors of Cocoa (*Theobroma cacao*) Yields in Ghana. *SustainableAgriculture Research*, 2(4), 117-128. <http://dx.doi.org/10.5539/sar.v2n4p117>
- Anonymous(2014). *Badan Koordinasi Penanaman Modal, Potensi Kakao di Sulawesi Barat* (Diakses tanggal 3 Juni 2014, Indonesian). Retrieved from <http://regionalinvestment.bkpm.go.id/newsipid/id/index.php>
- Azis, A.I., Rosmana, A., & Vien, S.D. (2013). Pengendalian Penyakit Hawar Daun *Phytophthora* pada Bibit

- Kakao dengan *Trichoderma asperellum*. *Jurnal Fitopatologi Indonesia*, 9(1), 15–20. Indonesian. <http://dx.doi.org/10.14692/jfi.10.5.139>
- Bae, H., Robert, D.P., Lim, H. S., Strem, M.D., Park, S. C., Ryu, C. M., ... Bailey, B.A. (2011). Endophytic *Trichoderma* Isolates from Tropical Environments Delay Disease Onset and Induce Resistance Against *Phytophthora capsici* in Hot Pepper Using Multiple Mechanisms. *Molecular Plant-Microbe Interactions*, 24(3), 336-351. <http://dx.doi.org/10.1094/MPMI-09-10-0221>
- Fosket, D.E. (1999). *Plant Growth and Development: A Molecular Approach*. Academic Press, San Diego, New York, London.
- Harman, G.E. (2011). *Trichoderma* -Not Just For Biocontrol Anymore. *Phytoparasitica*, 39, 103-108.
- Hermosa, R., Viterbo, A., Chet, I., & Monte, E. (2012). Plant-Beneficial Effects of *Trichoderma* and of Its Genes. *Microbiology*, 158, 17-25. <http://dx.doi.org/10.1099/mic.0.052274-0>
- Hopkins, W.G. (1999). *Introduction to Plant Physiology*. John Wiley and Sons, Inc., New York, Brisbane, Toronto.
- Nasaruddin (2011). *Bahan Ajar Mata kuliah Analisis Pertumbuhan Tanaman*. Universitas Hasanuddin, Makassar, Indonesian.
- Nurahmi, E., Susanna, & Sriwati, R. (2012). Pengaruh *Trichoderma* terhadap Perkecambah dan Pertumbuhan Bibit Kakao, Tomat, dan Kedelai. *J. Floratek*, 7, 57-65. Indonesian.
- Okoth, S.A., Otadoh, J.A., & Ochanda, J.O. (2011). Improved Seedling Emergence and Growth of Maize and Beans by *Trichoderma harziunum*. *Tropical and Subtropical Agroecosystem*, 13, 65-71. Retrieved from <http://hdl.handle.net/10568/43096>
- Ololade, I. A., Ajayi, I. R., Gbadamosi, A. E., Mohammed, O. Z., & Minggu, A. G. (2010). A Study on Effects of Soil Physico-Chemical Properties on Cocoa Production in Ondo State. *Modern Applied Science*, 4(5), 35-43. <http://dx.doi.org/10.5539/mas.v4n5p35>
- Saba, H., Vibhash, D., Manisha, M., Prashant, K. S., Farhan, H., & Tauseef, A. (2012). *Trichoderma* a Promising Plant Growth Stimulator and Biocontrol Agent. *Mycosphere*, 3(4), 524–531.
- Worldatlas (2015). *Top 10 Cocoa Producing Countries* (Published: April 22, 2015; Updated: July 14, 2016). Retrieved from <http://www.worldatlas.com/articles/top-10-cocoa-producing-countries.html>.

### Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).