Improvement of Health and Vigor of Hybrid Cacao (*Theobroma cacao* L.) Seedling through Incorporation of *Trichoderma* spp. in Seed Matriconditioning and Planting Media

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INTRODUCTION

- Our study in hybrid cacao seed showed that there were 13 species of fungal pathogens reducing seed vigor and cacao seedling (Baharudin *et al.* 2012).
- Application of seed treatment using matriconditioning plus *Trichoderma harzianum* DT/38 dan *T. pseudokoningii* DT/39 improved viability and vigor of hybrid cacao seed (Baharudin *et al.* 2010). This invigoration treatment using matriconditioning plus biological agents has been defined as biomatriconditioning (Ilyas and Sopian 2013).
- Use of planting media consist of soil:sand:organic matter (2:1:1) plus *Trichoderma* spp. is good for growth of coffea and cacao seedlings (Prawoto 2008).

OBJECTIVES

The objective of this study was to evaluate the effectiveness of seed biomatriconditioning and planting media using *Trichoderma* spp. in improving vigor of hybrid cacao seedlings.

MATERIALS AND METHODS

Seeds of hybrid cacao TSH 858 x Sca 6 were extracted from physiological matured fruits harvested at 150 days after anthesis from Seed Orchard of Research Center for Coffea and Cacao, Jember, Indonesia.

Compost was made from waste of cacao endocarp with *Trichoderma harzianum*, *T. pseudokoningii*, *Aspergillus niger*, and *Pholiota* sp. as composting activators. Fertilizers (N:P:K = 2:1:2) were provided at the rate of 1, 2, 3, 4, and 5 g/seedling for 1, 2, 3, 4, and 5 month old-seedlings, respectively.

This experiment was conducted using completely randomized factorial designed with four replications.The first factor was seed treatment (untreated, matriconditioning plus *T. harzianum* DT/38 and *T. pseudokoningii* DT/39), and the second factor was planting media (soil, soil:sand 2:1, soil:compost 2:1, soil:sand:compost 2:1:1, soil:compost 2:1 plus *T. harzianum* DT/38 + *T. pseudokoningii* DT/39, and soil:sand:compost 2:1:1 plus *T. harzianum* DT/38 + *T. pseudokoningii* DT/39). Matriconditioning was done by using ratio of seeds to carrier (burned rice hull 250 µ) to water of 4:2:1 for 5 h in 24 ^{0}C room. Cacao seeds (120 g) were wetted with 30 ml water containing *Trichoderma* spp. (10^6 spores/ml), then 60 g of the carrier was added and mixed thoroughly. Biomatriconditioned seeds were then planted directly.

RESULTS AND DISCUSSIONS

The result showed that biomatriconditioning was effective in increasing stem diameter, leaf area, root dry weight, and P content of leaves as compared to untreated. Planting media treatment of soil, sand and compost mixture (2:1:1) plus biological agents increased stem diameter, leaf area, root dry weight, P-K content of leaves of cacao hybrid seedlings better than other treatments (Table 1 and 2). There was no significant difference between biomatriconditioning and untreated seed if planted on this planting media treatment on seedling height, seedling dry weight, and N content in the leaves of 5 month-old cacao seedlings (Table 1).

Table 1. Effect of interaction between seed treatment and planting media on seedling height, seedling dry weight, nitrogen and phosphor leaf content of 5 month-old hybrid cacao seedling

Planting Media	Seed Treatment	
	Untreated	Biomatricon-ditioning
	Seedling height (cm)	
S	25.70 Bc	42.05 Aa
SS	32.48 Abc	38.13 Aa
SC	34.85 Ab	37.45 Aa
SSC	34.98 Ab	40.83 Aa
SCB	37.78 Aab	41.45 Aa
SSCB	42.00 Aa	43.02 Aa
	Seedling dry weight (g)	
S	10.51 Bc	13.13 Aa
SS	10.21 Bc	14.14 Aa
SC	11.20 Bc	13.51 Aa
SSC	11.74 Bb	14.03 Aa
SCB	13.94 Aa	13.73 Aa
SSCB	13.68 Aa	14.90 Aa
	Nitrogen leaf content (%)	
S	2.41 Bbc	2.55 Aa
SS	2.46 Aab	2.54 Aa
SC	2.37 Bc	2.54 Aa
SSC	2.55 Ba	2.44 Ab
SCB	2.52 Aab	2.61 Aa
SSCB	2.56 Aa	2.62 Aa
	Phosphor leaf content (%)	
S	0.51 Bb	0.55 Ab
SS	0.49 Bb	0.55 Ab
SC	0.52 Aa	0.57 Ab
SSC	0.48 Bb	0.55 Ab
SCB	0.48 Bb	0.62 Aa
SSCB	0.52 Ba	0.64 Aa

Note: S = soil, SS = soil + sand, SC = soil + compost, SSC = soil + sand + compost, SCB = soil + compost + biological agents, SSCB = soil + sand + compost + biological agents. Mean separation at 5% level (DMRT); capital letters within planting media values, small letters within seed treatment values.

Table 2. Effect of seed treatment and planting media on stem diameter, leaf area, root dry weight, and potassium leaf content

Planting Media	Seed Treatment		Average of Planting Media
	Untreated	Biomatri-conditioning	
	... Stem diameter (cm) ...		
S	0.26	0.35	0.31 c
SS	0.30	0.34	0.32 c
SC	0.28	0.39	0.33 bc
SSC	0.33	0.39	0.36 bc
SCB	0.34	0.43	0.39 ab
SSCB	0.39	0.44	0.42 a
AST	0.32 b	0.39 a	
	... Leaf area (cm^2) ...		
S	415.02	499.83	457.42 c
SS	397.02	503.44	450.23 c
SC	421.85	529.55	475.70 bc
SSC	421.92	505.46	463.69 bc
SCB	460.96	545.21	503.08 b
SSCB	506.55	607.50	557.02 a
AST	437.22 b	531.83 a	
	... Root dry weight (g) ...		
S	3.34	4.88	4.11 c
SS	3.40	4.65	4.03 c
SC	4.06	4.60	4.33 bc
SSC	3.94	4.70	4.32 bc
SCB	4.35	4.95	4.65 ab
SSCB	4.28	5.48	4.88 a
AST	3.89 b	4.88 a	
	... Potassium leaf content (%) ...		
S	4.02	3.52	3.77 b
SS	4.01	3.69	3.85 b
SC	3.97	3.94	3.95 b
SSC	3.85	4.21	4.03 b
SCB	3.90	4.44	4.17 ab
SSCB	4.45	4.67	4.56 a
AST	4.03 a	4.08 a	

Note: Details of planting media as in Table 1. Mean separation at 5% level (DMRT); letters within average of seed treatment (AST), and average of planting media values for each parameters.

Figure 1. Five month-old hybrid cacao seedlings derived from the seed treated with biomatriconditioning (B_1) and planted in soil (M_1) or soil + sand + compost + biological agents (M_6).

CONCLUSIONS

Matriconditioning plus *T. harzianum* DT/38 and *T. pseudokoningii* DT/39 applied on hybrid cacao seeds could replace the biological agent treatment in the planting media mixture of soil, sand and compost (2:1:1) to improve vigor, and N, P, K leaf content of 5 month-old seedlings.

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Turkey, June 16th, 2013

Seed Matriconditioning plus Natural or Synthetic Bactericides Eradicated Seedborne Bacterial Leaf Blight and Improved Viability and Vigor of Rice Seed

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INTRODUCTION

In Indonesia, 60% yield loss in rice due to *Xanthomonas oryzae* pv. *oryzae* (Xoo), a seedborne pathogen causing bacterial leaf blight (BLB).

Figure 1. Symptom of BLB on rice leaf

Seed invigoration treatment such as matriconditioning integrated with natural pesticides has been proved in overcoming seedborne pathogens while improving vigor in chili seed (Ilyas, 2006).

OBJECTIVES

The objectives of this study were (1) to find kind and concentrations of natural or synthetic bactericides to control Xoo in rice seed, and (2) to evaluate effect of matriconditioning plus natural or synthetic bactericides on viability and vigor and presence of Xoo in rice seed.

MATERIALS AND METHODS

Three experiments were conducted. First experiment was identification of Xoo. Second experiment was conducted to evaluate the effectiveness of natural bactericides either clove (*Syzygium aromaticum*) oil (a.i. eugenol 35%) or fragrant grass (*Andropogon nardus*) oil (a.i. citronella 35%) 0%, 0.5%, 1%, 1.5%, 2%; and synthetic bactericides (Agrept 20 WP, a.i. streptomycin sulphate 20%, Plantomycin 7 SP, a.i. streptomycin sulphate 7%, Nordox 56 WP, a.i. cupric oxide 56%) 0%, 0.1%, 0.2%, 0.3%, 0.4% on controlling Xoo. Then the effective concentrations of each kind were tested for phytotoxicity on the rice seeds. In third experiment, seed treatments were applied on rice seeds infected by Xoo.

RESULTS AND DISCUSSIONS

Results of experiment 1

Identification using Koch Postulate

Symptoms of BLB disease were shown after 14 day-old healthy rice seedling inoculated with Xoo isolated from seeds of cv. IR-64 and cv. Ciherang.

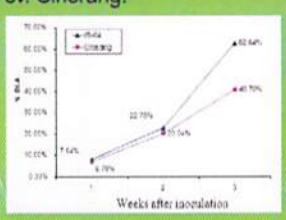

Figure 1. Increasing percent of disease leaf area in cv. IR-64 and cv. Ciherang up to 3 weeks after inoculation (using Koch Postulate).

Identification using Gram Method

Pure isolates of Xoo from rice seeds cv. IR-64 and cv. Ciherang indicated Xoo with microscopic signs of red or light red (Gram-negative), *bacillus* or *cocoid* at juvenile stage.

Results of experiment 2 indicated that the most effective (in preventing *in-vitro* growth of Xoo) and non toxic (not reducing seed viability) concentration for natural bactericide was fragrant grass oil 1%, and for synthetic bactericide was Agrept 0.2%.

Figure 2. Effect of natural bactericides on *in-vitro* growth of *Xanthomonas oryzae* pv. *oryzae*: clove oil 1% (left) and fragrant grass oil 1% (right).

Table 1. Effect of synthetic bactericide (Agrept 20 WP) concentrations on percent of germination, index of vigor, and speed of germination of rice seeds cv. IR-64 and cv. Ciherang

Parameters	Concentrations				
	0%	0.1%	0.2%	0.3%	0.4%
			cv. IR-64		
Germination (%)	76.0 b	83.0 ab	88.0 a	86.0 ab	83.0 ab
Index of vigor (%)	66.5 b	75.5 ab	78.5 a	78.0 a	73.5 ab
Speed of germination (%/d)	24.9 b	26.8 ab	29.1 a	26.8 ab	24.6 b
			cv. Ciherang		
Germination (%)	84.0 b	82.0 b	94.0 a	88.0 ab	89.5 ab
Index of vigor (%)	72.0 b	78.0 ab	86.5 a	79.0 ab	81.0 ab
Speed of germination (%/d)	27.9 c	29.8 bc	33.3 a	32.1 ab	31.4 ab

Note: Mean separation at 5% level (DMRT); letters within consentration values for each parameters.

Experiment 3 proved that matriconditioning plus natural or synthetic bactericides (fragrant grass oil 1 % or Agrept 20 WP 0.2%) improved seed physiological quality (increased germination, index of vigor, normal seedling dry weight, and reduced T_{50}) and pathological quality (eradicated Xoo) (Table 2).

Table 2. Effect of seed treatment applied on rice seeds cv. IR-64 and cv. Ciherang on percent of germination (G), index of vigor (IV), speed of germination (SG), normal seedling dry weight (NSDW), T_{50}, and population of Xoo (P)

Seed Treatments	Parameters				
	G (%)	IV (%)	NSDW (g)	T_{50} (d)	P (cfu)
			cv. IR-64		
Untreated	74.0 d	60.0 c	0.61 c	6.7 a	51.00 a
Agrept 0.2%	82.5 c	78.0 b	0.66 c	5.5 c	3.75 c
Fragrant grass oil (FGO) 1%	76.5 d	57.5 c	0.65 c	6.2 b	5.25 c
Matriconditioning (M)	95.0 a	85.0 a	0.85 a	4.5 d	33.50 b
M + Agrept 0.2%	92.5 ab	87.5 a	0.81 a	4.4 d	0.00 d
M + FGO 1%	87.5 bc	82.5 ab	0.75 b	4.6 d	0.00 d
			cv. Ciherang		
Untreated	76.0 d	60.5 c	0.61 d	6.7 a	40.0 a
Agrept 0.2%	85.0 c	80.5 b	0.69 c	5.4 c	3.0 c
Fragrant grass oil (FGO) 1%	79.5 d	59.0 c	0.65 cd	6.0 b	4.0 c
Matriconditioning (M)	94.5 ab	88.5 a	0.86 a	4.4 d	29.5 b
M + Agrept 0.2%	96.0 a	90.0 a	0.83 a	4.2 d	0.0 d
M + FGO 1%	90.0 bc	84.0 ab	0.76 b	4.4 d	0.0 d

Note: Mean separation at 5% level (DMRT); letters within seed treatment values for each parameters.

CONCLUSIONS

Incorporation of natural (fragrant grass oil a.i. citronella 35%) 1% or synthetic bactericides (Agrept 20 WP a.i. streptomycin sulphate 20%) 0.2% in matriconditioning not only improved rice seed viability and vigor but also eradicated the seedborne *Xanthomonas oryzae* pv. *oryzae*.

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