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In Vitro Selection of Abaca for Resistance to *Fusarium oxysporum* f.sp. *cubense*

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Abaca (*Musa textilis* Nee) is an important industrial crop. However, the cultivation of this crop in Indonesia is hampered by *Fusarium* wilt (Panama disease) as a result of *Fusarium oxysporum* f.sp. *cubense* (*Foc*) infection. The objectives of this study were to (i) evaluate inhibitory effects of culture filtrates (CF) of three *Foc* isolates (Banyuwangi, Malang, and Bojonegoro isolates) on shoot growth of abaca cv. Tangongon and Sangihe-1, (ii) determine sublethal concentration of *Foc* CF, (iii) isolate variant cells/tissues which are insensitive against *Foc* CF and regenerate plantlets from the variants, and (iv) evaluate responses of the plantlets against *Foc* infection. The results of the experiment showed that even though CF of all *Foc* isolates inhibited abaca's shoot growth, CF of *Foc* Banyuwangi isolate showed the most inhibitory effect. Sublethal concentration of CF of *Foc* Banyuwangi isolate was 40%. From abaca cv. Tangongon, 326 shoots were regenerated from CF insensitive embryogenic calli while from Sangihe-1 - 176 shoots were regenerated. Following acclimatization and *Foc* inoculation using detached-leaf dual culture test, a total of four immune, two resistant, and two moderately resistant plantlets were identified out of 45 tested variants of Tangongon. On the other hand, only two resistant and one moderately resistant plantlets were identified out of 10 tested variants of Sangihe-1.

Key words: *Fusarium* wilt, *in vitro* selection, culture filtrate, ethylmethanesulphonate (EMS), *Musa textilis* Nee

INTRODUCTION

In the previous experiments, screening of abaca (*Musa textilis* Nee) germplasm responses and evaluation of effective *Fusarium oxysporum* Schlecht f.sp. *cubense* (E.F. Smith) Snyd & Hans (*Foc*) inoculation methods have been conducted (Purwati 2006) as part of efforts to develop *Foc*-resistance abaca. Out of ten abaca cultivars tested, nine were identified as very susceptible, and one was susceptible to *Foc* infection (Purwati 2006).

Infection of *Foc* has been associated with *Fusarium* wilt (Panama disease) and has caused serious damages to abaca plantation in Indonesia (Nasir & Jumjunidang 2003). The existance of *Foc* in various regions in Indonesia hamperred abaca plantation development because of unavailability of *Foc*-resistance abaca cultivar (Damayanti 2004). Therefore, development of *Foc*-resistance abaca has become a major research topic at Central Research of Tobacco and Fibre Crops, Malang-Indonesia.

Genetic diversity of abaca tends to be low since it was vegetatively propagated. Increasing genetic diversity of vegetatively propagated crops could be done by induction of somaclonal variation (Ahloowalia & Maluszynski 2001). Moreover, *in vitro* selection might be used to screen somaclonal variants and identify ones having certain desirable characters, as it was reported in peanut (Yusnita *et al.* 2005). Induction of somaclonal variation and screening using *in vitro* selection have also been used to obtain *Fusarium* spp. resistance of carnation (*Dianthus caryophyllus*), wheat (*Triticum aestivum*), soybean (*Soja max*), pineapple (*Ananas comosus*), sugarcane (*Saccharum officinarum*), and vanilla (*Vanilla planifolia*) (Ahmed *et al.* 1996; Jin *et al.* 1996; Hidalgo *et al.* 1999; Yunus *et al.* 2000; Borras *et al.* 2001; Thakur *et al.* 2002; Inayati 2003).

The success of *in vitro* selection depended on at least two factors, i.e (i) the availability of efficient plant tissue culture techniques capable of regenerating large number of plantlets and at the same time capable of inducing somaclonal variation and (ii) the availability of *in vitro* selection methods capable of inhibiting growth of normal cells/tissues and of proliferating variant/mutant cells/tissues exhibiting certain desirable characters into plantlets (Yusnita *et al.* 2005). Compositions of tissue culture medium for regenerating large number of abaca plantlets have been developed (Mariska & Sukmadjaja 2003). Culture filtrates (CF) of *Foc* have also been proven effective as selective agents for screening *Foc* resistance variants of abaca, carnation, and vanilla (Thakur *et al.* 2002; Inayati 2003; Damayanti 2004).

General objectives of this research were to develop effective methods of *in vitro* selection using *Foc* CF and identify *Foc*-resistance abaca variants. The specific objectives were to (i) evaluate inhibitory effects of CF of three *Foc* isolates against growth of abaca shoots, (ii) determine sublethal concentration of CF of selected *Foc* isolate, (iii) regenerate abaca variant cells/tissues insensitive against CF of *Foc* into plantlets, and (iv) evaluate responses of regenerated abaca variants against *Foc* infection.

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MATERIALS AND METHODS

Preparation of *Foc* CF. Banyuwangi, Bojonegoro, and Malang isolates of *Foc* were isolated from field grown abaca showing symptoms of *Foc* infection. Fungal isolates used in this experiment have been isolated using single fragment of *Foc* hyphae. The procedures of *Foc* isolation have been reported previously (Purwati 2006). The *Foc* isolates were grown on potato dextrose agar (PDA) medium and incubated for seven days at 29-30 ^{o}C. Mycellia of *Foc* grown on PDA were inoculated into 250 ml of culture flasks containing 100 ml of potato dextrose broth (PDB) medium and the cultures were shaken on a shaker at 60 rpm for 14 days for *Foc* CF production. Harvested fungal cultures were sterilized by autoclaving at 121 ^{o}C for 20 minutes, micellia were separated from CF using miracloth, and it was used as selective agent.

Inhibitory Effects of *Foc* CF. Inhibitory effects of CF of three *Foc* isolates were evaluated by adding 10, 20, 30, 40, 50, and 60% (v/v) of the respective CF into selective abaca shoot induction (SI) medium. The abaca SI medium consisted of MS basal medium (Murashige & Skoog 1962) supplemented with 0.5 mg/l of BAP and 100 mg/l of ascorbic acid. The SI medium without addition of *Foc* CF (0%) was used as control.

In vitro propagated abaca cv. Tangongon and Sangihe-1 shoots (2-3 cm height) were planted on selective SI media. Evaluated abaca shoots were subcultured twice onto fresh selective SI medium during three months evaluation period. They were incubated in incubation room with 25 $\pm$ 2 ^{o}C temperature and 1000 lux illumination intensity for 16 hours. Experimental units consisted of a single shoot grown on a culture tube and for each evaluated treatment was repeated 20 times.

Observations were recorded after three months on percentage of shoot survival and score of damaged shoot (SDS). The SDS was determined according to criteria developed by Epp (1987), such as: score 0 = shoots were healthy, green and their leaves did not show any yellowing symptom; score 1 = shoots remained green but their growth were retarded, and lower leaves showed yellowing symptom; score 2 = basal of the shoots started to rot while shoot growth was retarded, and more leaves showed yellowing symptoms and the color of newly emmerged leaves were yellowish green; score 3 = basal of the shoots rot while shoot growth was retarded, and all leaves have showed yellowing symptoms; score 4 = shoots have completely rot and died (Figure 1a-e).

***In Vitro* Selection Using *Foc* CF.** A mutagenic treatment using ethylmethanesulphonate (EMS) was conducted by dipping 100 embryogenic calli of abaca cv. Tangongon and Sangihe-1 (size 3 x 3 x 3 mm^3) in 0.6% (w/v) EMS solution. The EMS treated embryogenic calli were shaken on rotary shaker at 100 rpm for two hours. After the EMS treatment, calli were rinsed once with sterilised distilled water and grown on abaca embryogenic callus proliferation (CP) medium.

The CP medium consisted of MS basal medium supplemented with 5 mg/l BAP, 0.4 mg/l thidiazuron [TDZ], and 100 mg/l ascorbic acid (Mariska & Sukmadjaya 2003; Purwati 2006). Abaca embryogenic calli were propagated for six months on CP medium to obtain at least 150 pieces of embryogenic calli (3 x 3 x 3 cm^3) and used as explants for *in vitro* selection on SI medium containing 40% (v/v) of CF of *Foc* Banyuwangi isolate (*Foc* Bw). The explants were subcultured three times onto fresh selective SI medium in a six months period. Observations were conducted after six months of *in vitro* selection on survival of explants, number of explants regenerating shoot, and number of regenerated shoots per explant.

Regeneration and Evaluation of Abaca Variants. Identified CF insensitive variants of abaca embryogenic calli as a results of *in vitro* selection were grown on SI medium without addition of *Foc* CF and regenerated shoots (> 2 cm in height) were rooted on root inducing (RI) medium. The RI medium consisted of MS basal medium supplemented with 1 g/l activated charcoal. Developed variants of abaca plantlets (rooted shoots) were acclimatized and grown in glasshouses. Responses of the variants against *Foc* Bw infection were evaluated indirectly using detached-leaf dual culture test (Pratt 1996). Seven pieces of leaf (1 x 1 cm^2) from each of the abaca variant were inoculated with *Foc* Bw hyphae and incubated on agar medium without nutrients (8 g/1 of agar) for 12 days. Leaf pieces of field grown plants of abaca cv. Tangongon and Sangihe-1 were also inoculated with *Foc* Bw and used as control.

Percentage of leaf pieces showing symptoms of *Foc* Bw infection and observed score of damaged leaf symptoms (SDL) were used to calculate disease intensity. Criteria of SDL were as follow: score 0 = no browning (necrotic) symptom on leaf pieces, score 1 = necrotic symptom less than 25%, score 2 = necrotic symptom between 25-50%, score 3 = necrotic symptom 50-75%, and score 4 = necrotic symptom > 75%, leaf pieces have completely rotten. Disease intensity (DI) was calculated using the following equation:

$$DI=[S(ni \times si)/(N \times S)] \times 100\%$$

ni: number of leaf pieces showing SDL = i, *si* - ith value of SDL; N - total number of evaluated leaf pieces, and S - the highest value of SDL (Cachinero *et al.* 2002). The responses of tested abaca variants against *Foc* Bw infection were concluded based on the value of calculated DI. The tested variants were identified as immune (Im) if DI = 0%; resistance (Rs) = if 0% < DI < 5%; moderately resistance (Mr) = if 5% < DI < 10%; moderately susceptible (Ms) = if 10% < DI < 25%; susceptible (Sc) = if 25% < DI < 50%; very susceptible (Vs) = if DI > 50% (Yusnita & Sudarsono 2004).

RESULTS

Inhibitory Effects of *Foc* CF. A total of 8 and 21% of shoots of abaca cv. Tangongon and Sangihe-1 were died when they were cultured on selective medium containing CF of *Foc* Bw. Addition of CF of *Foc* Bw at 10-30% resulted in percentage of dead shoots ranged from 8-40% (abaca cv. Tangongon) and 21-62% (abaca cv. Sangihe-1). On the otherhand, the rate of dead shoots of abaca cv. Tangongon and Sangihe-1 on selective SI medium containing 40-60% of CF of *Foc* Bw ranged from 90-100% (Table 1). None of abaca cv. Tangongon nor Sangihe-1 shoot died on SI medium without addition of CF of *Foc* Bw. The average of SDS on medium without addition of CF of *Foc* Bw for abaca cv. Tangongon and Sangihe-1 was 0.1 and 0, respectively.

Figure 1. Representative of abaca shoots exhibiting various score of damaged shoot symptoms of 0 to 4 (based on criteria developed by Epp 1987) after they were planted on selective medium containing Banyuwangi isolate of *F. oxysporum* f.sp. *cubense* culture filtrate.

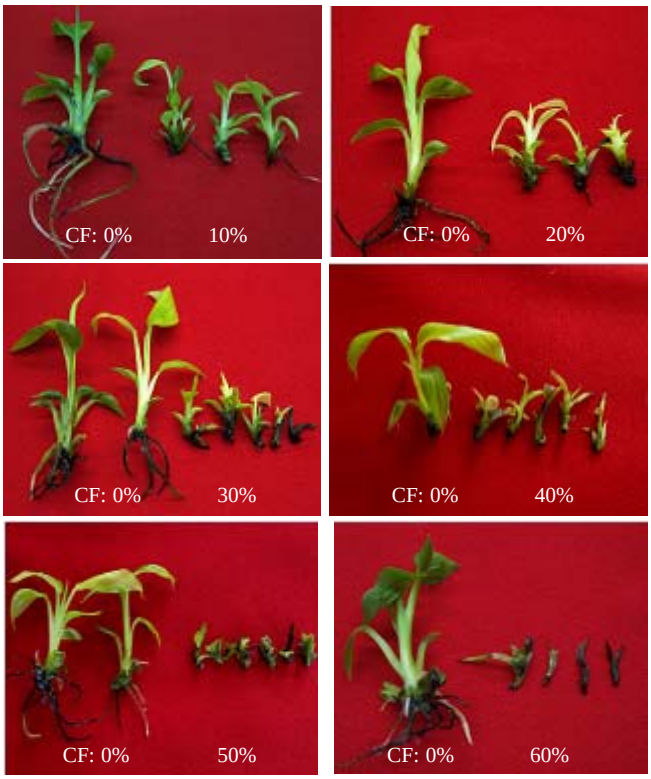

Figure 2. Inhibitory effects of Banyuwangi isolate of *F. oxysporum* f.sp. *cubense* (*Foc* Bw) culture filtrate (CF) on growth and shoot proliferation of abaca cv. Tangongon. Shoot responses on medium without CF of *Foc* Bw (CF 0%) and on selective medium containing 10, 20, 30, 40, 50, or 60% of CF of *Foc* Bw. Observations were recorded three months after culturing abaca shoots on selective media.

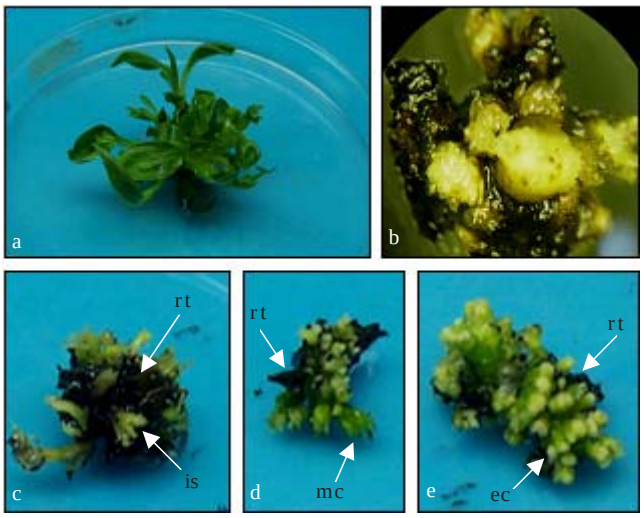

Figure 3. *In vitro* selection of abaca embryogenic calli on selective medium containing Banyuwangi isolate of *F. oxysporum* f.sp. *cubense* (*Foc* Bw) culture filtrate (CF). a. Embryogenic calli (EC) of abaca cv. Tangongon proliferating shoots on medium without CF of *Foc* Bw (control); b. *In vitro* selected EC were mostly necrosis, dormant (did not regenerate shoots), and in a few part proliferate shoots; c. shoots; d. rossete shoots (multiple bud clumps); and e. embryogenic calli-insensitive against CF as a result of in vitro selection on selective medium containing 40% of CF of *Foc* Bw. rt: rotten tissue, ec: embryogenic calli, is: CF insensitive shoots, mc: multiple bud clump.

Culture filtrate of either Malang (*Foc* Ml) or Bojonegoro (*Foc* Bn) isolate of *Foc* showed less inhibitory effects than that of Banyuwangi isolate. Dead shoots of abaca cv. Tangongon were only observed when they were cultured on

Table 1. Inhibitory effects of culture filtrate (CF) of Banyuwangi, Malang, or Bojonegoro isolates of *F. oxysporum* f.sp. *cubense* (*Foc*) on shoot growth and development of abaca cv. Tangongon and Sangihe-1. Observations were recorded three months after abaca shoots were cultured on selective medium containing CF of *Foc*.

Abaca cultivars and CF concentration (%)	PDS on media containing CF of *Foc*:			SDS on media containing CF of *Foc*:		
	Bw	Ml	Bn	Bw	Ml	Bn
Abaca cv. Tangongon:						
0	0	0	0	0.0aE*	0.1aD	0.0aF
10	8	0	0	1.7aD	0.3bD	0.0cF
20	15	0	0	2.0aC	0.8bC	0.4cE
30	40	0	0	3.2aB	1.0bC	1.2bD
40	90	37	0	3.9aA	3.2bB	1.7cC
50	94	75	40	3.9aA	3.8aA	3.2bB
60	100	83	87	4.0aA	3.9aA	3.9aA
Abaca cv. Sangihe-1:						
0	0	0	0	0.0aE	0.0aE	0.1aF
10	21	0	0	2.1aD	1.0bD	0.4cE
20	33	0	0	2.7aC	1.4bC	1.1cD
30	62	32	4	3.6aB	2.8bB	1.9cC
40	90	80	11	3.9aAB	3.9aA	2.1bBC
50	100	100	8	4.0aA	4.0aA	2.3bB
60	100	100	50	4.0aA	4.0aA	3.4bA

PDS: percentage of dead shoot (%); SDS: score of damaged shoot; CF: culture filtrate; Bw, Ml, Bn: are Banyuwangi, Malang, and Bojonegoro isolates of *Foc*. *Numbers on the same line followed by small letter or on the same column followed by capital letter-were not significantly different based on Duncan Multiple Range Test (α = 5%)

selective medium containing at least 40% (*foc* Ml) or 50% (*Foc* Bn) of the fungal filtrates. While for abaca cv. Sangihe-1, dead shoots were started to occur at addition of 30% (*Foc* Ml and Bn) of culture filtrates (Table 1). The recorded SDS data on CF of three different *Foc* isolates also indicated similar trend to that of shoot survival (Table 1).

Based on observed SDS data, abaca cv. Sangihe-1 tended to be more sensitive than that of Tangongon and CF of *Foc* Bw to be more inhibitory than that of *Foc* Ml or Bn, respectively. Concentration of CF of *Foc* Bw at 40 or 50% resulted in at least 90 or 94% of dead shoots in abaca cv. Tangongon and 90 or 100% of that in abaca cv. Sangihe-1, respectively. Therefore, addition of CF of *Foc* Bw at 40% was selected as sublethal concentration and used in subsequent experiments. Representative samples of inhibitory effects of various concentration of *Foc* CF added in selective SI medium on growth of abaca shoots were presented in Figure 2.

***In Vitro* Selection Using *Foc* CF.** Treatment of 0.6% EMS for two hours was conducted to induce cells/tissues mutation of abaca embryogenic calli. Although EMS treatment inhibited shoot proliferation, EMS treated embryogenic calli cultured on medium without addition of *Foc* CF proliferated on average of 19.8 (abaca cv. Tangongon) and 17.8 shoots/explant (abaca cv. Sangihe-1) (Table 2).

Success rates of *in vitro* selection of abaca cv. Tangongon and Sangihe-1 embryogenic calli using 40% of CF of *Foc* Bw were presented in Table 2. Most of the EMS treated abaca embryogenic calli cultured on selective SI medium containing 40% of CF rot and died. However, small fractions of explants grown on such selective medium proliferated embryogenic calli, rosette and normal shoots, respectively. The calli and shoots proliferated from surrounding rotten and dead tissues on selective SI medium were CF insensitive.

Embryogenic calli of abaca treated with 0.6% EMS for two hours and cultured on selective medium containing CF of *Foc* Bw were able to generate on average of 2.4 and 1.4 shoots/explant of abaca cv. Tangonon and Sangihe-1, respectively

Table 2. *In vitro* selection of EMS treated (0.6% for two hours) embryogenic calli of abaca on selective medium containing sublethal level (40%) of Banyuwangi isolate of *F. oxysporum* f.sp. *cubense* culture filtrate. Observations were recorded six months after embryogenic calli were cultured on selective medium

Parameters recorded	Variant of Tangongon on media:		Variant of Sangihe-1 on media:	
	Without CF	With CF	Without CF	With CF
Surviving explant:	88	137	88	126
Regenerating shoot (%)	99	11	99	19
Dormant (%)	1	21	1	13
Average number of shoots/explant	19.8	2.4	17.8	1.4
Number of shoots:				
< 2 cm	1454	231	1430	113
> 2 cm*	286	95	136	63
Total	1740	326	1566	176
Reduction of shoot number as compared to without CF (%)		88**		92**

*Shoots > 2 cm in height were ready for acclimatization. **Percentage of reduction of shoot number (%) was calculated using the following equation [(X0 x Y0 - X1 x Y1)/(X0 x Y0)] x 100%. X0 and Y0 were percentage of explants regenerating shoots and number of regenerated shoots per explant in control medium without *Foc* culture diltrate (CF), while X1 and Y1 were percentage of explants regenerating shoots and number of regenerated shoots per explant in selective medium with *Foc* CF

(Table 2). Representative samples of developing embryogenic calli after *in vitro* selection on medium conaining 40% of CF of *Foc* Bw were presented in Figure 3a-e.

In this experiment, *in vitro* selection has identified a total of 326 variant shoots of abaka cv. Tangongon and 176 variant shoots of abaca cv. Sangihe-1 from *Foc* Bw CF insensitive embryogenic calli (Table 2). Subsequently, the *Foc* Bw CF insensitive abaca variants were transferred into plastic pots (200 ml) containing sterile sands, acclimatized in room with controlled humidity (100%), and the surviving plants were grown in glasshouses.

Evaluation of Variant Abaca Shoots. Responses of variants of abaca cv. Tangongon against *Foc* infection using detached-

Table 3. Percentage of damaged leaf, average of score of damaged leaf, disease intensity, and resistance of variants derived from abaca cv. Tangongon and Sangihe-1. The abaca variants were regenerated from culture filtrate insensitive embryogenic calli as a result of in vitro selection on selective media containing Banyuwangi isolate of *F. oxysporum* f. sp. *cubense* (*Foc* Bw) culture filtrate. The variant responses were determined based on results of detached-leaf dual culture test using *Foc* Bw

Cultivars and variants of abaca	DL (%)	Average SDL	DI (%)	Resistance
Leaf of original Abaca:				
Field grown Tg	86	2.29	57	Vs
Field grown Sh	86	2.14	54	Vs
Leaf of *in vitro* selected plantlets: Abaca cv. Tangongon				
Tg 3.2.1.1-Foc3	0	0	0	Im
Tg 3.2.1.2-Foc3	0	0	0	Im
Tg 70.2.3-Foc5	0	0	0	Im
Tg 70.2.3-Foc7	0	0	0	Im
Tg 3.2.1.1-Foc2	14	0.14	4	Rs
Tg 3.2.1.1-Foc4	14	0.14	4	Rs
Tg 3.2.1.2-Foc4	29	0.29	7	Mr
Tg 70.2.3-Foc1	29	0.43	9	Mr
Leaf of *in vitro* selected plantlets: Abaca cv. Sangihe-1				
Sh 1.1.3-Foc4	14	0.14	4	Rs
Sh 1.1.3-Foc8	14	0.14	4	Rs
Sh 17.2.1-Foc7	14	0.29	7	Mr
Sh 1.1.3-Foc7	29	0.43	11	Ms
Sh 1.1.3-Foc10	29	0.43	11	Ms
Sh 4.1.1-Foc1	43	0.29	11	Ms
Sh 10.1.1-Foc2	43	0.57	14	Ms
Sh 17.2.1-Foc4	43	0.43	11	Ms
Sh 17.2.1-Foc3	100	3	75	Vs
Sh 20.2.2-Foc2	100	2.71	68	Vs

*Remarks: Im: immune, Rs: resistance, Mr: moderately resistance, Ms: moderately susceptible, and Vs: very susceptible, DL: percentage of damaged leaf, SDL: score of damaged leaf, DI: disease intensity, Tg and Sh: abaca cv. Tangongon and Sangihe-1

leaf dual culture test indicated among 45 variants tested, four plants were identified as immune since they did not show leaf damages due to *Foc* infection. In addition, two plants were identified as resistant and the other two were moderately resistant against *Foc* infection (Table 3). While for abaca cv. Sangihe-1, two *Foc* resistant and one moderately resistant plants were identified out of ten variants evaluated (Table 3). Results of detached-leaf dual culture test of the original abaca cv. Tangongon and Sangihe-1 indicated that they were very susceptible to *Foc* infection (Table 3).

DISCUSSION

This research was conducted to increase genetic diversity of abaca by EMS treatment. The treatment of abaca embryogenic calli with EMS was effective for inducing abaca variants since plantlets variants could be directly regenerated from the calli. Embriogenic calli could also subsequently be exposed to selective medium containing *Foc* CF to identify CF insensitive variants.

Regeneration from CF insensitive variant of abaca cells/tissues could result in *Foc* resistant abaca plantlets. Therefore, induced variation and *in vitro* selection approaches could be used as alternative routes for developing *Foc* resistance abaca cultivars. Induced variation with chemical mutagenic agents such as EMS, followed by *in vitro* selection have also been used to obtain certain desirable characters of vegetatively propagated crops (Maluszynski *et al*. 1995; Roux *et al*. 1999; Joseph *et al*. 2004).

Fusarium oxysporum f.sp. *cubense* is fungal pathogen infecting abaca and secrette nonhost specific toxin necessary for infection proccesses. The CF of *Foc* could be used as selective agent in the *in vitro* selection for identifying *Foc* resistance variants, as it was demonstrated in carnation, wheat, soybean, pineapple, sugarcane, and tomato (Toyoda *et al*. 1984; Fadel & Wenzel 1993; Ahmed *et al*. 1996; Jin *et al*. 1996; Hidalgo *et al*. 1999; Yunus 2000; Borras *et al*. 2001; Thakur *et al*. 2002; Inayati 2003).

Results of this research showed, CF from all of three tested *Foc* isolates (Banyuwangi, Bojonegoro, and Malang) exhibited different inhibitory effects on shoot growth of abaca cv. Tangongon and Sangihe-1. Culture filtrate of *Foc* Bw showed the highest inhibitory effects as compared to that of *Foc* Bn and *Foc* Ml. Differences in the inhibitory effects might be because of differences in the rates of toxin production by the respective isolates. The *Foc* Bw might be able to produce more toxin than that of *Foc* Ml or *Foc* Bn. Fungal isolates capable of producing more toxin have been shown to posses more inhibitory CF (Cachinero *et al*. 2002).

In addition to secretting toxin, *Foc* was also known to secrete plant growth regulator (PGR) such as auxin or gibberelin and secreting various secondary metabolites such as alkaloid, steroids, and terpenoid (Goodman *et al*. 1986; Rademacher 1994; Thrane 2001). The PGR secreted into *Foc* CF might have positive effects on proliferation of CF insensitive abaca cells/tissues. If it was calculated based only on surviving explants, recorded data showed that *Foc* CF insensitive embryogenic callus variants of abaca cv. Tangongon when cultured on selective SI medium regenerated similar number of shoots/explant to that of the control medium (data not presented).

Observed inhibitory effects of *Foc* CF on abaca embryogenic calli in this experiment supported previous reports on such CF effects on carnation (Thakur *et al*. 2002) and pineapple (Hidalgo *et al*. 1999). In these previous reports,

addition of CF of *Fusarium* spp. on selective medium inhibited growth and development of the explants. Previous results also stated that an increase in *Foc* CF concentration in selective medium resulted in less surviving explants (Li *et al*. 1999; Borras *et al*. 2001; Thakur *et al*. 2002).

Although EMS treated (0.6% for two hours) embryogenic calli of abaca cv. Tangongon and Sangihe-1 resulted in *Foc* CF insensitive embryogenic calli; not all of shoots regenerated from insensitive calli were resistance against *Foc* infection. The failure of getting *Foc* resistance shoots from abaca CF insensitive calli indicated the presence of chimaeric or escaped tissues in the calli. Chimaeric shoots might have grown from a mixture of normal (CF sensitive) and variants (CF insensitive) of abaca embryogenic calli. At the stage of shoot proliferation, chimaeric shoots were grown in medium without *Foc* CF and new shoots might have proliferated from normal (sensitive) tissues resulted in regeneration of *Foc* susceptible abaca shoots.

Based on results of this experiment, although CF of all *Foc* isolates inhibited abaca's shoot growth, CF of *Foc* Bw showed the most inhibitory effect. Sublethal concentration of CF of *Foc* Bw was 40%. From abaca cv. Tangongon, 326 shoots were regenerated from CF insensitive embryogenic calli while from Sangihe-1 - 176 shoots were obtained. Following acclimatization and *Foc* Bw inoculation using detached-leaf dual culture test, a total of four immune, two resistant, and two moderately resistant plantlets were identified out of 45 tested variants of abaca cv. Tangongon. On the other hand, only two resistant and one moderately resistant plantlets were identified out of ten tested variants of Sangihe-1.

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