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Resistance of Abaca Somaclonal Variant Against *Fusarium*

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The objectives of this study were (i) to evaluate responses against *F. oxysporum* f.sp. *cubense* (*Foc*) infection of abaca variants regenerated using four different methods, (ii) to determine initial root length and plant height effects on survival of inoculated abaca variants, and (iii) to identify *Foc* resistance abaca variants. In the previous experiment, four abaca variant lines were regenerated from (i) embryogenic calli (TC line), (ii) ethyl methyl sulphonate (EMS) treated embryogenic calli (EMS line), (iii) EMS treated embryogenic calli, followed by *in vitro* selection on *Foc* culture filtrate (EMS+CF line), and (iv) EMS treated embryogenic calli, followed by *in vitro* selection on fusaric acid (EMS+FA line). All abaca variants were grown in a glasshouse and inoculated with Banyuwangi isolate of *Foc* (*Foc* Bw). Initial root length (RL) and plant height (PH) of the abaca variants were recorded before inoculation, while scores of plant damage (SPD), and their survival were recorded at 60 days after inoculation (DAI). The results showed that the initial RL and PH did not affect survival of the tested abaca variants. Regardless of their initial RL and PH, susceptible abaca variants died before 60 DAI while resistance ones still survived. Abaca variants regenerated from single clump of embryogenic callus showed an array of responses against *Foc* Bw infection, indicating the existence of a mix cells population. The *Foc* Bw resistance abaca variants were successfully identified from four tested abaca variant lines, although with different frequencies. However, more *Foc* Bw resistance abaca plants were identified from EMS+CF line than the others. Using the developed procedures, 8 resistance abaca plants were identified from abaca cv. Tangongon and 12 from abaca cv. Sangihe-1.

Key words: *Fusarium* wilts resistance, *in vitro* selection, culture filtrate, fusaric acid, EMS

INTRODUCTION

Fusarium wilt due to *Fusarium oxysporum* Schlecht f.sp. *cubense* (E.F. Smith) Snyd and Hans (*Foc*) infection is one of diseases infecting abaca plantation in the tropical region. *Fusarium* wilt disease reduced yield of abaca plantation at Leyte, the Philiphines by as much as 5-65% (Bastasa & Baliad 2005). In Indonesia, the existence of this pathogen restricts the development of abaca plantation since there is no *Foc* resistance abaca cultivar as yet (Damayanti 2004).

Planting *Foc* resistance abaca is an alternative method for controlling *Foc* infection (http://www.plantmanagementnetwork.org). Induction of somaclonal variation and application of *in vitro* selection may be used to generate and identify *Foc* resistance abaca somaclonal variants. Moreover, mutagenesis and *in vitro* selection of mutagenized explants may effectively could be used to increase genetic variation of vegetatively propagated crops (Roux 2004), such as abaca. Hence, abaca variants with several certain superior characters could be identified among *in vitro* regenerated abaca plants. Induction of somaclonal variation and *in vitro* selection have been used to develop variant lines with resistance against a number of diseases (Ahmed *et al.* 1996; Jin *et al.* 1996; Hidalgo *et al.* 1999; Yunus 2000; Borras *et al.* 2001; Thakur *et al.* 2002).

Induced mutation by ethyl methyl sulphonate (EMS) treatment and *in vitro* selection on media containing *Foc* culture filtrate (CF) or fusaric acid (FA) have been used to increase somaclonal variation and isolate *Foc* resistant lines. Abaca variants were regenerated from embryogenic calli and from EMS treated embryogenic calli of abaca cv. Tangongon and Sangihe-1, respectively. Both *Foc* CF insensitive and FA insensitive abaca variants have also been obtained through *in vitro* selection on media containing *Foc* CF or FA (Purwati 2006). The objectives of this research were (i) to evaluate responses of abaca variants regenerated using four different methods against infection of Banyuwangi isolate of *Foc* (*Foc* Bw) in the glasshouse, (ii) to determine effects of initial root length (RL) and plant height (PH) on survival of *Foc* Bw inoculated abaca variants, and (iii) to identify *Foc* Bw resistance abaca plants.

MATERIALS AND METHODS

Regeneration of Abaca Variant Lines. Four tested abaca variant lines (TC, EMS, EMS+CF, and EMS+FA lines) were regenerated from abaca somatic embryos using four different methods. The TC line was regenerated directly from embryogenic calli while EMS line were regenerated from EMS treated embryogenic calli of abaca. The EMS+CF line was regenerated from CF insensitive abaca somatic embryos originated from EMS treated embryogenic calli, followed by *in vitro* selection on selective medium containing 30% of CF of *Foc* Bw. On the other hand, the EMS+FA line was regenerated from FA insensitive abaca somatic embryos originated from EMS treated embryogenic calli, followed by *in vitro* selection on medium containing 0.3% FA.

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Regeneration of respective abaca variant lines from embryogenic calli has been conducted in previous experiments (Purwati 2006).

Preparation of *Foc* Inoculum. The *Foc* Bw was grown on PDA medium and incubated in incubation room with 29-30 °C room temperature for seven days. Agar pieces with *Foc* Bw mycellia were inoculated onto 250 ml culture flask containing 100 ml of PDB medium. The cultures were shaken at 60 rpm for 14 days until they formed conidia. The fungal mycelia were removed using sterile nylon cloth to obtain stock of *Foc* conidia. The *Foc* conidial densities of the stock were counted by serial dilutions and conidial counting using haemocytometer (Gregory 1983). Subsequently, the conidial stock was diluted to 10^6 conidia/ml and used as inocullum.

Response of Individual Abaca Plant Against *Foc* Bw. This experiment was conducted to determine responses of individual plant of four abaca variant lines against *Foc* Bw infection. In this experiment, number of regenerated and tested individual plant from abaca cv. Tangongon and Sangihe-1 were as follows: for TC line were 5 and 4 plants, EMS line - 12 and 27 plants, while EMS+CF line - 17 and 25 plants, respectively (Table 1). For EMS+FA line, the number of regenerated and tested individuals were only eight plants from abaca cv. Tangongon, since no Sangihe-1 derived plant was available (Table 1). All tested abaca variants were grown in a glasshouse for up to three months. When they were evaluated against *Foc* Bw, initial height of the tested abaca plants was between 15-30 cm.

Effects of Initial RL and PH on Responses. This experiment was conducted to investigate effects of initial RL and PH on responses of tested abaca variants against *Foc* Bw infection. The initial RL of evaluated abaca variants were separated into five groups (Table 2 & 3), such as: RL-1 group with initial RL < 12.5 cm, RL-2 with 12.5 cm > RL ≥ 18.8 cm, RL-3 with 18.8 cm > RL ≥ 25.0 cm, RL-4 with 25.0 cm > RL ≥ 31.3 cm, and RL-5 with RL > 31.3 cm. The initial PH of evaluated abaca variants were also separated into five groups (Table 2 & 3), such as: PH-1 group with initial PH < 15.0 cm, PH-2 with 15.0 cm > PH ≥ 18.3 cm, PH-3 with 18.3 cm > PH ≥ 21.5 cm, PH-4 with 21.3 cm > PH ≥ 24.8 cm, and PH-5 with PH > 24.8 cm. All groups of tested abaca variants were grown in a glasshouse and evaluated for their response against *Foc* Bw infection.

Table 1. Response of individual plant belonging to four lines of abaca variants against infection of Banyuwangi isolate of *F. oxysporum* f.sp. *cubense* (*Foc* Bw). Observation were conducted at 60 days post inoculation

Lines and cultivar of abaca:	Number of tested abaca variants: *Foc* Bw inoculated	Died	Survived	Percentage of survival (%)
TC line*:				
Tangongon	4	3	1	25
Sangihe-1	5	4	1	20
EMS line:				
Tangongon	12	4	8	67
Sangihe-1	28	15	13	46
EMS+CF line:				
Tangongon	17	9	8	47
Sangihe-1	25	13	12	48
EMS+FA line:				
Tangongon	8	6	2	25
Sangihe-1	ND	ND	ND	ND

*TC line: abaca variants were regenerated directly from embryogenic callus; EMS line: from embryogenic callus treated with EMS; EMS+CF line: from embryogenic callus treated with EMS, followed by *in vitro* selection on medium containing *Foc* culture filtrate; EMS+FA line: from embryogenic callus treated with EMS, followed by *in vitro* selection on medium containing fusaric acid. ND: no data were obtained

Responses of Variants Originated from Single Explant. This experiment was conducted to determine whether a number of abaca plants regenerated from single embryogenic callus showed similar responses against *Foc* Bw infection. Using the developed regeneration and *in vitro* selection methods, two or more abaca variants were usually obtained from single callus. In this experiment, four number of variants from EMS line, three number from EMS+CF line, and one number from EMS+FA line of abaca cv. Tangongon were tested. Four number of variants from EMS and from EMS+CF lines were tested for abaca cv. Sangihe-1 (Table 4). All tested abaca variants were grown in a glasshouse and evaluated for their responses against *Foc* Bw infection.

Inoculation of Abaca Variant with *Foc* Bw. All abaca variants were tested for their responses by inoculating them with *Foc* Bw using procedures developed previously (Purwati 2006). Roots of tested abaca variants were injured by cutting and the plants were dipped for two hours on 250 ml of conidial suspension of *Foc* Bw. After dipping on conidial suspension of *Foc* Bw, the abaca variants were planted in polybag (15 x 30 cm) containing 3 kg of sterile mix of soil:sand:compost (2:1:1 v/v). Growth and percentage of survival of the inoculated variants were recorded for up to 60 days after inoculation (DAI). Score of plant damages (SPD) were recorded at 60 DAI and used as indicators of *Foc* Bw infection. Number of days when the inoculated abaca plants died were also noted. Score of plant damages due to *Foc* Bw infection was determined using criteria developed by Epp (1987) and used in previous experiment (Purwati 2006). Examples of abaca plants with various levels of SPD due to *Foc* Bw infection were presented in Figure 1a-e.

RESULTS

Abaca Variant Lines. Among five variants of TC line of abaca cv. Tangongon and four variants of TC line of abaca cv. Sangihe-1, only one variant did not die and still survived 60 DAI with *Foc* Bw. Eight and 13 variants did not die and still survived 60 DAI with *Foc* Bw among 12 variants of EMS line i.e. from abaca cv. Tangongon and 27 variants of Sangihe-1, respectively (Table 1).

Among 17 variants of abaca cv. Tangongon and 25 variants of cv. Sangihe-1 of EMS+CF line, 8 and 12 variants did not die and still survived 60 DAI with *Foc* Bw, respectively. On the other hand, among eight variants of abaca cv. Tangongon of EMS+FA line, only two plants did not die and still survive 60 DAI with *Foc* Bw (Table 1).

Response of Individual Variant Against *Foc* Bw. All variants from four abaca variant lines (Figure 1f) tested for their response against *Foc* Bw, none showed the value of SPD=0 (Figure 1a). However, a number of abaca variants with SPD=1 or 2 (Figure 1b-c) were identified among tested variants of TC, EMS, EMS+CF, and EMS+FA lines (Table 2 & 3).

Table 2. Effects of initial root length and plant height on number of days when the tested individual abaca cv. Tangongon variants died and score of plant damages at 60 days after inoculation with Banyuwangi isolate of *F. oxysporum* f.sp. *cubense* in the glasshouse

Lines and variants no.	Initial root length	Initial plant height	Score of plant damages	Days when the variant died
TC line:*				
Tg-6	RL-2	PH-2	4	42
Tg-12	RL-3	PH-4	4	14
Tg-8	RL-4	PH-4	4	42
Tg-7	RL-5	PH-5	4	42
Tg-9	RL-5	PH-5	1	- **
EMS line:				
Tg-25.5.1-4	RL-1	PH-2	2	-
Tg-70.2-4	RL-2	PH-3	4	28
Tg-59.2-4	RL-3	PH-3	4	14
Tg-45.2-7	RL-3	PH-2	4	28
Tg-25.5.1-5	RL-3	PH-3	2	-
Tg-59.2-5	RL-3	PH-4	2	-
Tg-59.2-7	RL-3	PH-4	2	-
Tg-59.2-8	RL-4	PH-5	2	-
Tg-70.2-8	RL-4	PH-5	2	-
Tg-70.2-9	RL-4	PH-5	2	-
Tg-45.2.1-1	RL-5	PH-3	4	14
Tg-3.1-2	RL-5	PH-5	1	-
EMS+CF line:				
Tg-3.2.1.2-4	RL-2	PH-3	2	-
Tg-70.2.3-5	RL-2	PH-4	1	-
Tg-45.2.2.2-6	RL-3	PH-3	4	28
Tg-70.2.3-3	RL-3	PH-4	4	28
Tg-70.2.3-2	RL-3	PH-4	4	56
Tg-70.2.3-4	RL-3	PH-4	4	56
Tg-3.2.1.2-3	RL-3	PH-4	2	-
Tg-45.2.2.2-2	RL-3	PH-4	1	-
Tg-3.2.1.2-5	RL-4	PH-3	4	14
Tg-45.2.2.1-3	RL-4	PH-4	4	14
Tg-45.2.2.2-4	RL-4	PH-2	4	14
Tg-45.2.2.2-3	RL-5	PH-3	4	14
Tg-3.2.1.1-2	RL-5	PH-4	4	56
Tg-3.2.1.1-3	RL-5	PH-5	2	-
Tg-3.2.1.1-4	RL-5	PH-4	2	-
Tg-70.2.3-1	RL-5	PH-5	1	-
Tg-70.2.3-7	RL-5	PH-4	1	-
EMS+FA line:				
Tg-70.3.1.1-3	RL-3	PH-4	4	28
Tg-70.3.1.1-2	RL-3	PH-5	2	-
Tg-70.3.1.1-6	RL-3	PH-4	2	-
Tg-70.3.1.1-10	RL-4	PH-4	4	14
Tg-70.3.1.1-8	RL-4	PH-5	4	14
Tg-70.3.1.1-9	RL-4	PH-4	4	14
Tg-70.3.1.1-7	RL-4	PH-4	4	28
Tg-70.3.1.1-4	RL-4	PH-4	4	56

*TC line: abaca variants were regenerated directly from embryogenic callus; EMS line: from embryogenic callus that has been treated with EMS; EMS+CF line: from embryogenic callus that has been treated with EMS, followed by *in vitro* selection on medium containing *Foc* culture filtrate; EMS+FA line: from embryogenic callus that has been treated with EMS, followed by *in vitro* selection on medium containing fusaric acid. **(-) indicated the tested abaca variants still survived and did not die 60 days after inoculation

Results of the experiment indicated that abaca variants with both SPD=1 and 2 initially showed wilting symptoms of *Foc* Bw infection. However, they were able to survive and did not die 60 DAI (Figure 1g). Based on that observation, abaca variants with SPD=1 or 2 were identified as variants with increased resistance against *Foc* Bw infection than the original abaca cultivars.

However, most of the tested abaca variants showed SPD=4 (Figure 1e), similar to that of the original abaca cv. Tangongon and Sangihe-1. Tested abaca variants with SPD=4 died prior to 60 DAI (Figure 1g), and their response against *Foc* Bw were regarded as similar to that of the original abaca cultivars. Distribution frequency of SPD of TC, EMS, EMS+CF, and EMS+FA abaca variants lines at 60 days after *Foc* Bw inoculation was summarized in Figure 2.

Effects of Initial RL and PH on Responses. There were a number of abaca variants belonging into RL-2 to RL-5 and PH-2 to PH-5 groups, after inoculation with *Foc* Bw showed either classified as SPD=1 or SPD=4 (Figure 3, Table 2 & 3). Such data indicated that differences in both initial RL and PH of the tested abaca variants did not effect on their responses against *Foc* Bw infection. The differences in the observed SPD values were depended more on the genetic make up of the tested variants and less on their initial RL or PH. After

Tabel 3. Effects of initial root length and plant height on number of days when the tested individual abaca cv. Sangihe-1 variants died and score of plant damages at 60 days after inoculation with Banyuwangi isolate of *F. oxysporum* f.sp. *cubense* in the glasshouse

Lines and variants no.	Initial root length	Initial plant height	Score of plant damages	Days when the variant died
TC line*:				
Sh-5	RL-1	PH-1	4	14
Sh-7	RL-3	PH-4	4	28
Sh-6	RL-4	PH-2	4	14
Sh-3	RL-5	PH-4	1	- **
EMS line:				
Sh-10.1-10	RL-2	PH-1	4	28
Sh-17.1.2-4	RL-2	PH-4	4	28
Sh-42.2-5	RL-2	PH-2	4	28
Sh-1.1.1-2	RL-2	PH-2	2	-
Sh-10.1-8	RL-2	PH-2	1	-
Sh-17.1.1-1	RL-2	PH-2	2	-
Sh-10.1-4	RL-3	PH-2	4	28
Sh-10.1-6	RL-3	PH-1	4	28
Sh-42.2-4	RL-3	PH-2	4	14
Sh-42.2-7	RL-3	PH-3	4	28
Sh-10.1-2	RL-3	PH-2	2	-
Sh-10.1-3	RL-3	PH-3	1	-
Sh-17.1.1-2	RL-3	PH-5	1	-
Sh-17.1.2-2	RL-3	PH-3	2	-
Sh-42.2-1	RL-3	PH-4	1	-
Sh-42.2-2	RL-3	PH-3	2	-
Sh-10.1-5	RL-4	PH-3	4	28
Sh-17.1.2-1	RL-4	PH-4	4	28
Sh-1.1.1-1	RL-4	PH-5	1	-
Sh-42.2-8	RL-4	PH-2	2	-
EMS+FK line:				
Sh-10.1.1-5	RL-1	PH-2	4	28
Sh-17.2.1-8	RL-1	PH-2	4	14
Sh-42.2.3-3	RL-1	PH-1	4	14
Sh-42.2.3-6	RL-1	PH-2	4	14
Sh-1.1.3-4	RL-2	PH-3	4	42
Sh-42.2.3-1	RL-2	PH-2	4	14
Sh-42.2.3-5	RL-2	PH-1	4	28
Sh-1.1.3-7	RL-2	PH-3	1	-
Sh-10.1.1-3	RL-2	PH-3	2	-
Sh-1.1.3-11	RL-3	PH-2	4	14
Sh-1.1.3-2	RL-3	PH-3	4	14
Sh-10.1.1-4	RL-3	PH-4	4	28
Sh-17.2.1-6	RL-3	PH-3	4	14
Sh-1.1.3-6	RL-3	PH-3	2	-
Sh-1.1.3-9	RL-3	PH-2	2	-
Sh-17.2.1-4	RL-3	PH-3	2	-
Sh-17.2.1-7	RL-3	PH-2	1	-
Sh-1.1.3-10	RL-4	PH-3	1	-
Sh-10.1.1-2	RL-5	PH-4	1	-

*TC line: abaca variants were regenerated directly from embryogenic callus; EMS line: from embryogenic callus that has been treated with EMS; EMS+CF line: from embryogenic callus that has been treated with EMS, followed by *in vitro* selection on medium containing *Foc* culture filtrate; EMS+FA line: from embryogenic callus that has been treated with EMS, followed by *in vitro* selection on medium containing fusaric acid. **(-) indicated the tested abaca variants still survived and did not die 60 days after inoculation

inoculation with *Foc* Bw, resistance abaca variants (SPD=1) regardless of their initial RL and PH, were able to survive at 60 DAI (Table 2 & 3). On the other hand, susceptible abaca variants (SPD=4), died before 60 DAI with *Foc* Bw regardless of their initial RL and PH (Table 2 & 3).

Responses of Variants from Single Explant. Although they were regenerated from single EMS treated embryogenic callus, a number of regenerated abaca variants did not consistently show the same responses against *Foc* Bw infection (Table 4). Similar results were also observed among abaca variants regenerated from single EMS treated and *in vitro* selected on medium containing FA (EMS+FA) or *Foc* CF (EMS+FA) embryogenic callus (Table 4, Figure 1h). Such data indicated that the variants were regenerated from a mixture of cells with different genetic make up and that the *in vitro* selection alone was not able to eliminate the cell mixtures.

DISCUSSION

Although we could not obtain abaca variants that completely free from *Foc* Bw infection, abaca variants with improved resistance against *Foc* Bw, showing SPD=1 or 2 and still survived 60 days after *Foc* Bw inoculation, were identified in this research. The improved resistance abaca variants still exhibited symptoms of *Foc* Bw infection at the early stage; however, at later stages the symptoms

Table 4. Response variations among individual abaca variant regenerated from single explant of embryogenic callus against infection of Banyuwangi isolate of *F. oxysporum* f.sp. *cubense*. Score of plant damages was observed at 60 days after inoculation

Code of embryogenic callus	No. of regenerated plants/callus	Number of variants with score of plant damages:		
		1	2	4
Abaca cv. Tangongon				
EMS line*:				
Tg-25.5.1	2	0	2	0
Tg-45.2	2	0	0	4
Tg-59.2	4	0	3	1
Tg-70.2	3	0	2	1
EMS+CF line:				
Tg-45.2.2	5	1	0	4
Tg-3.2.1	6	0	4	2
Tg-70.2.3	6	3	0	3
EMS+FA line:				
Tg-70.3.1	8	0	2	6
Abaca cv. Sangihe-1				
EMS line:				
Sh-1.1.1	2	1	1	0
Sh-10.1	7	2	1	4
Sh-17.1.1	5	1	2	2
Sh-42.2	6	1	2	3
EMS+CF line:				
Sh-1.1.3	7	2	2	3
Sh-10.1.1	4	1	1	2
Sh-17.2.1	4	1	1	2
Sh-42.2.3	4	0	0	4

*TC line: abaca variants were regenerated directly from embryogenic callus; EMS line: from embryogenic callus that has been treated with EMS; EMS+CF line: from embryogenic callus that has been treated with EMS, followed by *in vitro* selection on medium containing *Foc* culture filtrate; EMS+FA line: from embryogenic callus that has been treated with EMS, followed by *in vitro* selection on medium containing fusaric acid

Figure 1. Phenotype of abaca showing various score of plant damages. (a) score 0, (b) score 1, (c) score 2, (d) score 3, and (e) score 4 - adapted based on the criteria developed by Epp (1987). (f) Representative of abaca variants evaluated for their responses against Banyuwangi isolates of *F. oxysporum* f.sp. *cubense* (*Foc* Bw) infection in the glasshouse. (g) Abaca variants died or survived at 60 days after innoculation with *Foc* Bw. (h) Abaca variants regenerated from single embryogenic callus exhibited variation in responses against *Foc* Bw infection, ranging from left to right: no damage, partially wilted, to totally wilted (died) plants.

subsequently dissapeared and inoculated plants did not die. Based on their response against *Foc* Bw infection, abaca variants with SPD=1 or 2 might probably have the ability to gradually activate the defensive mechanisms. In such case, when it was inoculated with *Foc* Bw, the tested resistance abaca variants would show infection symptoms at early stage, the symptoms would gradually dissapeared as the activation of defensive mechanisms progressed, and eventually they were recovered from *Foc* Bw infection.

Since the original of abaca cv. Tangongon and Sangihe-1 used in this research were very susceptible against *Foc* Bw (Purwati 2006), the resistance mechanisms among abaca variants might be acquired during proliferation period of the abaca embryogenic calli. These possibilities were supported

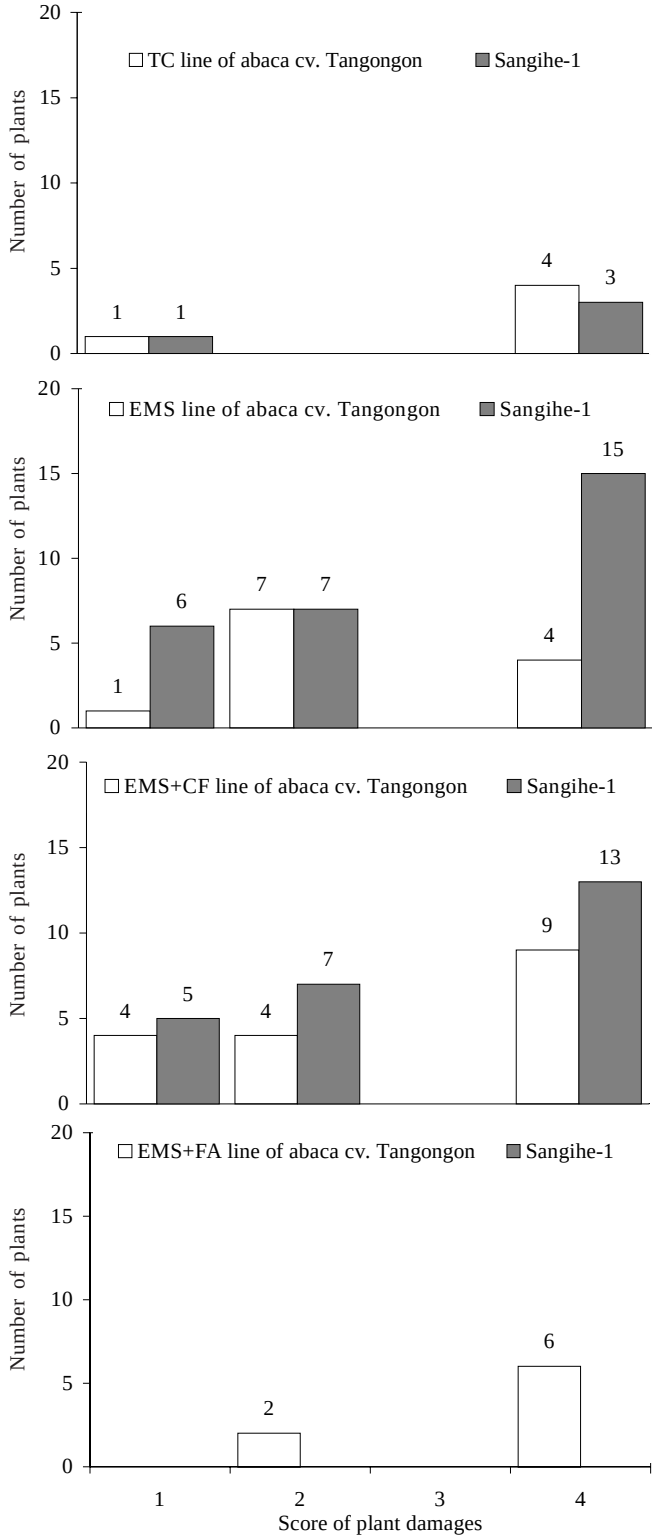

Figure 2. Distribution of responses based on score of plant damages (SPD) at 60 days after inoculation with Banyuwangi isolate of *F. oxysporum* f.sp. *cubense*. The evaluated abaca variants were regenerated from tissue culture, EMS treated, EMS treated followed by *in vitro* selection on medium containing *Foc* culture filtrate or fusaric acid - of embryogenic calli.

by identification of one *Foc* Bw resistance variant among five tested variants of TC line.

The collected data in this research indicated that mutagenesis with EMS treatment increased probability of

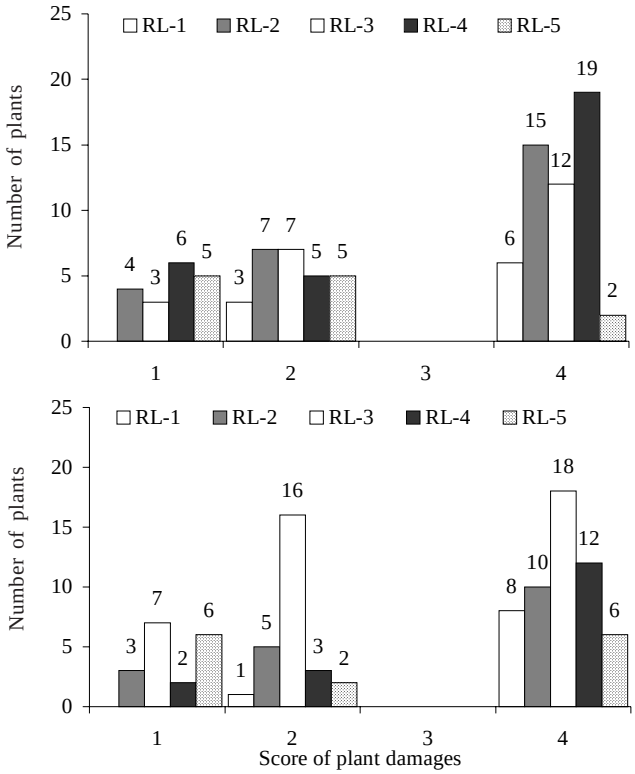

Figure 3. Effects of initial plant height (PH) and root length (RL) on distribution frequencies of score of plant damage at (a) 30 and (b) 60 days after inoculation of abaca variants with Banyuwangi isolate of *F. oxysporum* f.sp. *cubense*. Total evaluated abaca variants were 99 plants, consisted of those regenerated from tissue culture, EMS treated, EMS treated followed by *in vitro* selection on medium containing *Foc* culture filtrate or fusaric acid - of abaca embryogenic calli.

getting *Foc* Bw resistance abaca variants. Frequency of obtaining *Foc* Bw resistance abaca variants from EMS line was higher than that of TC line. On the other hand, *in vitro* selection on selective medium containing FA or *Foc* CF only slightly increased the frequency of obtaining *Foc* resistance abaca variants.

In vitro selection only screened for the desirable variants of cells/tissues and did not increase the frequency of mutations. Data from this research support that statement since the frequency of getting *Foc* Bw resistance abaca variants from EMS and EMS+CF lines were similar. However, the effectiveness and benefit of using only EMS treatment or EMS treatment followed by *in vitro* selection on medium containing FA could not be concluded in this research since number of evaluated individuals of abaca variants were small. More abaca variants from those lines need to be further evaluated to determine effectiveness of *in vitro* selection on medium containing FA. Nevertheles, data in this research indicated that *in vitro* selection on medium containing *Foc* CF is more effective than that containing FA. This conclusion was in line with the fact that there were a number of phytotoxins existed in *Foc* CF in addition of FA, such as trichothecenes (Svabova & Lebeda 2005).

In its pathogenicity processes, *Foc* synthesizes and secrettes fusaric acid that reduces activity of phenol and polyphenol oxydase enzymes and disturbes defensive

mechanisms of the plant host (Svabova & Lebeda 2005; Bouizgarne *et al*. 2006). In this research, *Foc* resistance abaca variants were regenerated from both FA or *Foc* CF insensitive embryogenic calli. Therefore, one possible mechanism improve *Foc* resistance among the identified variants could be through toxin detoxification. Natural resistance mechanisms against fungal pathogen secretting FA is generally through FA degradation at the early process of infection (Svabova & Lebeda 2005). A number of enzymes were reported to be able to degrade FA, such as: peroxydase and phenylalanine ammonia-lyase (PAL) (He *et al.* 2002). However, further investigations need to be conducted to prove that detoxyfication was actually the mechanism existed among *Foc* Bw resistance abaca variants yielded in this research.

Based on the results, it can be concluded that initial RL and PH did not affect abaca variant responses against infection of *Foc* Bw. Susceptible abaca plants eventually died before 60 DAI with *Foc* Bw. On the other hand, resistance abaca variants still survived even after 60 DAI, regardless of their initial RL and PH. A number of abaca variants regenerated from single embryogenic callus did not consistently exhibited similar responses against *Foc* Bw inoculation, indicating the existance of various cells/tissues with different genetic make up within the embryogenic callus.

Although the percentages were different, *Foc* Bw resistance abaca variants were identified among four abaca variant lines tested in this experiment. However, *Foc* Bw resistance abaca variants were obtained more frequently from EMS+CF line. With the developed procedures, eight *Foc* Bw resistance variants were identified from abaca cv. Tangongon and 12 from Sangihe-1.

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