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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.

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.

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

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).