View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

HAYATI Journal of Biosciences, March 2008, p 1-7 Vol. 15, No. 1
ISSN: 1978-3019

Inoculation Methods and Conidial Densities of *Fusarium oxysporum* f.sp. *cubense* in Abaca

RULLY DYAH PURWATI[1], NURUL HIDAYAH[1], SUDJINDRO[1], SUDARSONO[2*]

[1]Research Institute of Tobacco and Fiber Crops, Jalan Raya Karangploso, P.O. Box 199, Malang 65000, Indonesia
[2]Plant Molecular Biology Lab, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Jalan Meranti, Darmaga Campus, Bogor 16680, Indonesia

Received September 7, 2006/Accepted November 12, 2007

Abaca (*Musa textilis* Nee) is an important industrial crop. Its cultivation in Indonesia is, however, hampered by the wilt (Panama disease) caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) infections. Developing *Foc* resistance abaca lines require availability of established and reliable screening methods for resistance against *Foc*. The objectives of this study were to evaluate the (i) effectiveness of *Foc* inoculation methods, (ii) extent of *Foc* conidial densities – for causing the wilt in abaca, and (iii) responses of ten abaca cultivars against *Foc* infection. Results of this study showed that the method of inoculation by wounding abaca roots followed submerging the wounded plant in suspension of *Foc* conidia (10^6 conidia/ml) for 2 hours before planting was the most effective method for causing the wilt. Among ten abaca cultivars tested, none was resistant to *Foc* infection. Based on the calculation of disease intensity, nine abaca cultivars were identified as very susceptible, where as one cultivar was susceptible to *Foc* infection.

Key words: Fusarium wilt, panama disease, disease response, screening method, *Musa textilis*

INTRODUCTION

Fusarium wilt, also known as Panama disease, is one of the diseases causing significant yield reduction in plantation of *Musa* spp. in the tropics, including abaca (*Musa textilis* Nee). *Fusarium oxysporum* Schlecht. f.sp. *cubense* (E.F. Smith) Snyd and Hans has infected banana plantations across Asia, Africa, Australia, and tropical regions of America since 50 years ago (Hwang & Ko 2004). At Leyte, Philipines, *F. oxysporum* f.sp. *cubense* (*Foc*) infection has caused 5-65% damages among abaca plantations in the field (Bastasa & Baliad 2005). In Indonesia, *Foc* has been reported to infect banana plants at three provinces in Sumatera by as large as 3,300 ha (Nasir & Jumjunidang 2003). Indications of the existence of this pathogen at other regions in Indonesia was also recorded. Such conditions hampered large scale abaca plantations since *Foc* resistance cultivar was not available.

Control of *Foc* infection in the field is difficult since the pathogen is able to survive for such a long period of time in the form of mycelium among infected plant debris or in the form of chlamydospora in soil (Agrios 1997). Integrated disease control has been suggested as strategy for managing Fusarium wilt, such as: planting healthy and *Foc* resistance abaca cultivars, employing antagonistic microbes, and applying botanical pesticides (Djatnika *et al*. 2003; Di Pietro *et al*. 2003). However, some studies indicated the ability of antagonistic microbes to control *Foc* was still limited, its effectiveness in the field has not been proven, and there has not been any report identifying effective and reliable antagonistic microbes against *Foc* (Bastasa & Baliad 2005). So far, it was suggested that planting *Foc* resistant abaca cultivar was the most effective method for controlling *Foc* in banana (http://www.plantmanagementnetwork.org/pub/php/management/bananapanama).

Planting *Foc* resistance abaca cultivar might also be the most effective strategy for controlling *Foc* infection in Indonesia. Therefore, screening abaca germplasms resisting *Foc* infection is necessary as a part of efforts for identifying *Foc* resistance abaca. The success of germplasm identification is depended in part on the availability of effective methods of *Foc* inoculation and screening *Foc* resistance abaca. The available screening methods were impractical in their implementation (Ishak & Dwimahyani 2005). Therefore, the objectives of this experiment were to evaluate the (i) effectiveness of *Foc* inoculation methods; (ii) effects of *Foc* conidial densities – for causing Fusarium wilt in abaca; and (iii) responses of ten abaca cultivars against *Foc* infection.

MATERIALS AND METHODS

Plant Materials and *Foc* Isolates. The *Foc* was isolated from abaca showing symptoms of *Foc* infection obtained from PT. Bayu Lor Abaca Estate, Banyuwangi (isolate Bw), from Research Institute of Tobacco and Fiber Crops (Balittas)- Field Experiment Station at Sumberejo, Bojonegoro (isolate Bn) and from Karangploso, Malang (isolate Ml). Fungal isolates used in these experiments have been isolated from culture of *Foc* grown in potato dextrose agar (PDA) medium and incubated at 29-30 °C for seven days. Three innoculla of this culture were inoculated into 250 ml of culture flask containing 100 ml of potato dextrose broth (PDB) medium. The cultures were shaken on a shaker at 60 rpm for 14 days (Figure 1a). The fungal mycelia were removed using sterile nylon cloth to obtain stock of *Foc* conidia. The density of conidial

*Corresponding author. Phone: +62-251-377557, Fax: +62-251-629347, E-mail: prof_sudar@yahoo.com

Copyright © 2008 Institut Pertanian Bogor. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

suspension was counted using haemocytometer (Gregory 1983) and the desirable densities was obtained by diluting the stock using sterile water.

Rooted and *in vitro* propagated plantlets of abaca 'Tangongon' were prepared for evaluating the effectiveness of inoculation methods. Plantlets were individually planted in a plastic pot containing sterile sands (200 g per pot) and acclimatized in a controlled room with 100% humidity and 1,000 lux illumination intensities for one week. After acclimatization period, the surviving plantlets were transferred into a plastic pot (15 x 30 cm) containing 3 kg of sterile soil medium (a mixture of soil:sand:compost = 2:1:1 v/v) and grown in a glasshouse for two months (plant height will reach approximately 15-20 cm). The healthy plants were used later in this experiment (Figure 1b). The abaca 'Tangongon' and three different isolates of *Foc* (Bw, Bn, Ml) were used to evaluate the effects of inoculation methods, while three abaca cultivars ('Tangongon', 'Sangihe-1', and 'UB-3') and one *Foc* isolate (Bw) were used to evaluate those of inocullum density. As many as 10 abaca cultivars ('Banjar', 'BL Manado', 'Cilacap', 'Cirebon', 'Layahan', 'MbB', 'Sangihe-1', 'Sangihe-2', 'Tangongon', and 'UB-3') derived from *in vitro* culture were used for evaluating responses of abaca cultivars to *Foc* infection.

Effectiveness of *Foc* Inoculation Methods. In this experiment, the effectiveness of three inoculation methods and three *Foc* isolates (Bw, Bn, and Ml) were evaluated against abaca 'Tangongon'. For inoculation method 1 (INO-1), two months old of abaca 'Tangongon' was planted in sterile soil medium (3 kg of mixtures of soil:sand:compost = 2:1:1 v/v) that has been inoculated with fungal cultures. The fungal cultures were obtained by soaking 50 g of rice on sterile water for 24 hours, followed by inoculating the rice with mycelia of the respective *Foc* isolates grown on PDA medium, and by incubating the cultures at 29-30 ^{o}C for seven days. The ratio of soil medium to fungal cultures was 300:1 v/v (3 kg of soil medium: 10 g of fungal preparation). The mixing of sterile soil medium with fungal cultures was conducted seven days before planting of abaca. For inoculation method 2 (INO-2), root tips of two months old of abaca 'Tangongon' were cut 1 cm and wounded plants were dipped for two hours in 25 ml of suspension of *Foc* conidia (10^6 conidia/ml). Subsequently, inoculated abaca plants were planted on sterile soil medium as described above. Meanwhile for inoculation method 3 (INO-3), two month old of abaca 'Tangongon' was planted in sterile soil medium as described previously. Subsequently, each of the potting medium was drenched with 50 ml of *Foc* conidial suspension (10^6 conidia/ml). Two month old abaca 'Tangongon' planted in sterile potting medium as described previously without *Foc* inoculation was used as control (INO-0).

Experiments were set up in a complete randomized design. The tested abaca 'Tangongon' were planted individually in a plastic pot. Experimental unit consisted of five abaca plants and each treatment consisted of four replicates. Therefore, 20 abaca plants (5 plants per unit x 4 replications) were evaluated for each treatment combination with a total of 12 treatment combinations (4 methods of inoculation x 3 different isolates). There were a total of 240 plants tested in this experiment. Observation was conducted to determine the percentage of plants showing symptoms of *Foc* infection, the average of score of wilting symptoms, and the disease intensity at 30 days after inoculation (DAI). Statistical data analysis was conducted only for the average of score of wilting symptoms.

Effects of Conidium Densities on Disease Intensities. The objective of this experiment was to evaluate the extent of conidium densities for causing symptoms of *Foc* infection and wilt disease intensity on three abaca cultivars ('Tangongon', 'Sangihe-1', and 'UB-3'). The evaluated conidial densities of Banyuwangi isolate of *Foc* were 10^5 and 10^6 conidia/ml. These densities were obtained by diluting stocks of conidial suspension using sterile water. The amount of dilution depended on the densities of harvested conidial stock.

Prior to *Foc* inoculation, root tips of the evaluated plants were cut 1 cm using sterile scissors. Each of the injured plant was dipped for two hours in 25 ml of *Foc* conidial suspension according to treatments (0, 10^5, and 10^6 conidia/ml). Subsequently, *Foc* inoculated abaca 'Tangongon', 'Sangihe-1', and 'UB-3' were planted individually in plastic pots containing sterile soil medium as previously described. Uninoculated abaca plants were used as control. The experimental unit consisted of three abaca plants and each treatment consisted of three replicates. Therefore, 9 abaca plants (3 plants per unit x 3 replications) were evaluated for each treatment combination with a total of 9 treatment combinations (3 conidium densities x 3 abaca cultivars). There were a total of 27 plants tested in this experiment for each abaca cultivar. Observation was conducted to determine the percentage of plants showing symptoms of *Foc* infection, the average of score of wilting symptoms, and the disease intensity at both 30 and 60 DAI. In addition, the occurrences of necrotic symptom at the base of inoculated abaca plants was observed at 60 DAI.

Response of 10 Abaca Cultivars Against *Foc* Infection. The objectives of this experiment were to evaluate occurrences of symptoms, the score of wilting symptoms and the disease intensity on ten abaca cultivars ('Banjar', 'BL Manado', 'Cilacap', 'Cirebon', 'Layahan', 'MbB', 'Sangihe-1', 'Sangihe-2', 'Tangongon', and 'UB-3') after inoculation with Banyuwangi isolate. The experimental unit consisted of four abaca plants and each treatment consisted of three replications. Therefore, 12 abaca plants (4 plants per unit x 3 replications) were evaluated for each treatment (with or without inoculation) and a total of 24 plants were tested for each abaca cultivar.

Prior to *Foc* inoculation, root tips of evaluated abaca were cut 1 cm using sterile scissors. Each of the injured plant was dipped for two hours in 25 ml of suspension of *Foc* conidia (10^6 conidia/ml). Subsequently, the *Foc* inoculated abaca was planted individually in a plastic pot containing sterile soil medium as previously described. Uninoculated abaca plants were used as the control. Observation was conducted at 60 DAI.

Scoring of Wilting and Necrotic Symptoms and Disease Intensities. Scoring of wilting symptoms in tested abaca due to *Foc* infection (score 0-4) was conducted following criteria

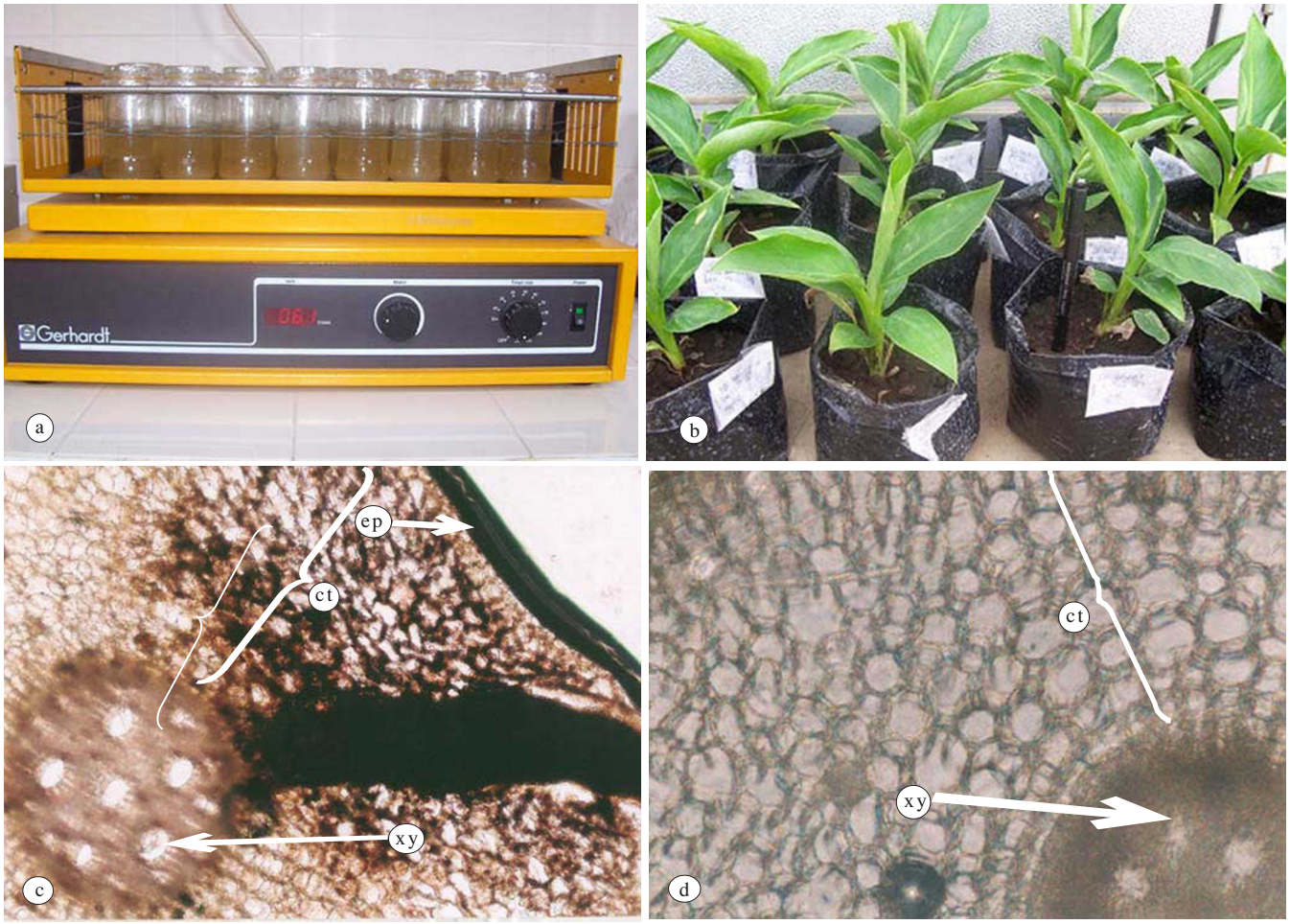

Figure 1. a. Fungal cultures of *F. oxysporum* f.sp. *cubense* (*Foc*) on potato dextrose broth medium; b. Two months old abaca plants used in the experiments and ready for testing against *Foc*; c. Photomicrograph of transversal cut of abaca roots at 30 days after inoculation with *Foc* (200x). Dark brown colour on epidermal and cortex tissues indicated root damages; d. transversal cut of healthy abaca roots (uninoculated root). ep: epidermal tissue, ct: cortex, and xy: xylem tissues.

developed by Epp (1987), such as: score 0 – inoculated plants were healthy and did not show wilting symptoms; score 1 – a number of leaves at lower part of plants yellowing; score 2 – number of yellowing leaves increased and the inoculated abaca plants started showing symptoms of wilting; score 3 – all plant parts were necrosis except for the new and unopened leaves; score 4 – the tested abaca plants died (Figure 2a-e). Necrotic symptoms at the base of the abaca plants were indicated by changing in tissue colors from greenish white to dark brown or black. Scoring of necrotic symptoms at the base of *Foc* inoculated abaca plants was conducted as follows: score 0 – no changes in tissue colors; score 1 – the symptoms ranged between 0-5%; score 2 – the symptoms ranged between 5-35%; score 3 – the symptoms ranged between 35-50%; score 4 – the symptoms ranged between 50-75%; and score 5 – the symptoms > 75% (Mak *et al*. 2004b).

Disease intensity (DI) based on the wilting or the necrotic symptoms was calculated using the following equation:

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

ni: number of abaca plants with i^{th} score of symptoms, si: the value of the i^{th} score of symptoms, N: total number of tested abaca plants, and S: the highest value of score of symptoms (Cachinero *et al*. 2002).

Figure 2. Phenotypes of abaca plants with various degrees of wilting symptoms at 60 days after inoculation with *F. oxysporum* f.sp. *cubense*. a. Score 0, b. Score 1, c. Score 2, d. Score 3, and e. Score 4, based on criteria developed by Epp (1987).

Grouping responses of the tested individual abaca plant after *Foc* inoculation was conducted using the following criteria: immune (Im) – if the value of DI is equal to 0%; resistant (Rs) – if 0 > DI ≥ 5%; moderately resistant (Mr) – if 5% > DI ≥ 10%; moderately susceptible (Ms) – if 10% > DI ≥ 25%; susceptible (Sc) – if 25 > DI ≥ 50%; and very susceptible (Vs) - if DI > 50% (Yusnita & Sudarsono 2004).

Root Histology. Twenty abaca roots were sampled randomly from *Foc* inoculated abaca plants at 30 DAI. The roots were rinsed with tap water and fixed with 60% of formaline:alcohol (70%):glacial acetic acid (FAA, 5:90:5). Three transversal section (ca. 0.01 cm) were taken from every root sample using a razor blade. The damages of root tissues due to *Foc* infection were observed using a microscope. The presence of dark brown or blackenned tissues indicated tissue damages as suggested by Nasir *et al*. (2003).

RESULTS

Effectiveness of *Foc* Inoculation Methods. The control plants did not show wilting symptoms, while inoculated ones showed various degrees of wilting at 30 DAI. The colonies of *Foc* were successfully re-isolated from inoculated abaca showing the symptoms, indicating association between the symptoms and *Foc* infection.

Compared to other inoculation methods the INO-2 method yield the highest disease intensity and score of the symptoms (Table 1). In addition, INO-2 method was the only method consistently causing 100% of plant showing wilting symptoms (Table 1). Banyuwangi (Bw), Bojonegoro (Bn), and Malang (Ml) isolates were all able to infect and cause the symptoms on abaca using INO-2 method. On the other hand, when using either INO-1 or INO-3 method, the tested *Foc* isolates were not able to induce symptoms of *Foc* infection in several abaca plant tested.

Effects of *Foc* Conidium Densities. The *Foc* inoculation by dipping the plants at 10^5 or 10^6 conidia/ml were equally able to cause the wilting symptoms on the three tested abaca cultivars. At 30 DAI, the infected abaca showed that the score of the symptoms was lower than two (Table 2). Both at 30 and 60 DAI, inoculation with 10^6 conidia/ml resulted in higher percentages of abaca plants showing the symptoms, the score of wilting symptoms, and disease intensities than that with 10^5 conidia/ml except for 'Tangongon' at 30 DAI (Table 2). At 60 DAI, inoculation with 10^6 conidia/ml resulted in 100% of abaca 'Tangongon' and 'Sangihe-1' showing the symptoms. On the other hand inoculation with 10^5 conidia/ml only resulted in 100% of the plants showing the symptoms in 'Tangongon'.

Inoculation with 10^6 conidia/ml also resulted in higher percentages of necrotic symptoms and disease intensities than that with 10^5 conidia/ml (Table 3). However, statistical analysis indicated that the average of necrotic symptoms between the two conidial densities were not significantly different.

Histological observations at 30 DAI indicated that *Foc* inoculated abaca plants showed damages on epidermal and cortex tissues of root, as indicated by the presence of dark brown colors in evaluated samples (Figure 1c). However, the damages due to *Foc* infection has not reached xylem tissues. Transversal sections of *Foc* uninoculated abaca root in the control was presented in Figure 1d.

Phenotypes of abaca plants at 60 DAI showing wilting symptom scores of 0 – 4 were presented in Figure 2a-e. Those figures were used as references for scoring the symptoms of the tested plants.

Response of 10 Abaca Cultivars to *Foc* Infection. Uninoculated abaca plants did not show wilting symptoms due to *Foc* infection. After Bw inoculation, percentages of abaca plant showing the symptoms on ten abaca cultivars

Table 1. Effects of inoculation methods of three *F. oxysporum* f.sp. *cubense* isolates on percentage of plants showing wilting symptoms of *Foc* infection, score of wilting symptoms, and disease intensities of abaca 'Tangongon'. Observation was conducted at 30 days after inoculation

Inoculation methods	Plant showing wilting symptoms (%)			Score of wilting symptoms				Disease intensities (%)		
	Bw	Bn	Ml	Bw	Bn	Ml	Average	Bw	Bn	Ml
INO-0	0	0	0	0	0	0	0c*	0	0	0
INO-1	73	100	100	1.5	1.0	1.8	1.4b	37	25	45
INO-2	100	100	100	2.0	2.0	2.0	2.0a	50	50	50
INO-3	100	60	100	1.6	1.0	1.4	1.3b	40	25	35

*Numbers followed by the same letter were not significantly different based on Duncan Multiple Range Test with an α = 5%. Bm, Bn, ML were Banyuwangi, Bojonegoro, and Malang isolates of *Foc*, respectively.

Table 2. Effects of conidial densities of Banyuwangi isolate (Bw) of *F. oxysporum* f.sp. *cubense* on percentage of plant showing wilting symptoms of *Foc* infection, score of wilting symptoms, and disease intensities of abaca 'Tangongon' (Tg), 'Sangihe-1' (Sh), and 'UB-3' (Ub)

Days of observation and densities of conidia per ml	Plant showing wilting symptoms (%)			Score of wilting symptoms				Disease intensities (%)		
	Tg	Sh	Ub	Tg	Sh	Ub	Average	Tg	Sh	Ub
30 days after Bw inoculation:										
0 (control)	0	0	0	0	0	0	0b*	0	0	0
10^5	56	22	33	1.3	0.7	0.3	0.7a	33	17	8
10^6	44	67	67	1.3	1.7	1.0	1.3a	33	42	25
60 days after Bw inoculation:										
0 (control)	0	0	0	0	0	0	0c	0	0	0
10^5	100	89	44	2.3	1.7	1.1	1.7b	58	42	28
10^6	100	100	89	3.3	3.7	3.0	3.3a	83	92	75

*Numbers followed by the same letter were not significantly different based on Duncan Multiple Range Test with an α = 5%.

Table 3. Effects of conidial densities of Banyuwangi isolate (Bw) of *F. oxysporum* f.sp. *cubense* on plant showing necrotic symptoms, average score of necrotic symptoms, and disease intensities of abaca 'Tangongon' (Tg), 'Sangihe-1' (Sh), and 'UB-3' (Ub). Observations were conducted at 60 days after inoculation

Conidial densities (conidia/ml)	Plant showing necrotic symptoms (%)			Score of necrotic symptoms				Disease intensities (%)		
	Tg	Sh	Ub	Tg	Sh	Ub	Average	Tg	Sh	Ub
0 (control)	0	0	0	0	0	0	0b*	0	0	0
10^5	56	88	67	2.8	2.8	1.8	2.4a	56	56	36
10^6	78	100	56	3.2	4.1	2.7	3.3a	64	82	53

*Numbers followed by the same letter were not significantly different based on Duncan Multiple Range Test with an $\alpha = 5\%$.

Table 4. Responses of 10 abaca cultivars against Banyuwangi isolate of *F. oxysporum* f.sp. *cubense* infection. The responses were recorded at 60 days after inoculation

Cultivars and characters of abaca	Plant with wilting symptoms (%)	Score of wilting symptoms		Disease intensities (%)		Response against *Foc*
		INO	No-INO	INO	No-INO	
'Banjar' PH > 2 m, FR > 3%	90	2.2a*	0.8b*	55.0	20.0	Vs
'BL Manado' PH > 2 m, PR > 70%	100	3.5a	0.9b	87.5	22.5	Vs
'Cilacap' PH > 3 m, FR > 3%	100	2.8a	1.2b	65.0	30.0	Vs
'Cirebon' PH > 2 m, FR > 3%	100	3.9a	0.4b	97.5	10.0	Vs
'Layahan' NS > 25	80	2.1a	0.8b	52.5	20.0	Vs
'MbB'	90	1.6a	0.8a	40.0	20.0	Sc
'Sangihe-1' PH > 2 m, PR > 70%	100	3.7a	0.8b	93.0	20.0	Vs
'Sangihe-2' PH > 2 m, FR > 3%	100	3.5a	0.6b	87.5	15.0	Vs
'Tangongon'	100	3.3a	0.8b	82.0	20.0	Vs
'UB-3'	85	3.0a	0b	75.0	0	Vs

*For each abaca cultivar, numbers followed by the same letter in inoculated (INO) and without inoculation (No-INO) treatments were not significantly different based on Duncan Multiple Range Test with an $\alpha = 5\%$. Descriptions of the abaca cultivars were made according to Setyo-Budi *et al*. (2001); Vs: very susceptible; Sc: susceptible; PH: plant height; NS: number of suckers; FR: Fiber ratio; PR: paper yield ratio. Uninoculated abaca plants did not show wilting symptoms due to *Foc* infection.

were ranged from 80-100% (Table 4). All tested abaca cultivars showed higher average of score of wilting symptom and disease intensities than the control (Table 4). Based on disease intensities, ten abaca cultivars tested were idenified as either very susceptible (9 cultivars) or susceptible (1 cultivar).

DISCUSSION

The success of *Foc* infection in banana are the final results of a complex processes involving at least five stages, namely: (i) recognition by *Foc* of signals released by banana roots, (ii) *Foc* attachment on the banana root surface and penetration of *Foc* hyphae into root tissues, (iii) penetration of *Foc* hyphae into cortex tissues and degradation of physical defense system of root tissues, (iv) proliferation of *Foc* hyphae and production of microconidium in xylem tissues, and (v) secretion of fungal toxin and hydrolytic enzimes by *Foc* resulting in further tissue damages of the infected roots (Di Pietro *et al*. 2003). Wounding of root tissues assisted initial process of *Foc* infection in banana. Infection of *Foc* in abaca might also through similar processes and wounding might also assisted *Foc* infection.

In the field, banana plant tissues might be wounded because of talled trees due to strong wind, prunning of suckers, damages due to sucking insects or nematode infection activities (http://www.plantmanagementnetwork. org/pub/ php/management/ bananapanama). Djatnika *et al*. (2003) stated that *Foc* is a soil-borne pathogen. It infects banana plants through lateral root hairs, and is able to infect root tissues through wounding due to infection of *Radopholus similis* nematode. Numbers of ways resulting wound in banana plants may also happens in abaca and they might also be the initial routes of *Foc* to infect abaca in the field.

Foc inoculation using INO-2 method resulted more plants showing *Foc* infection symptoms and higher disease intensity than the inoculation using other methods. Such data were inline with *Foc* infection processes in abaca requiring the presence of damages in root tissues for initial *Foc* collonization. Wounding of abaca root tissues followed by dipping of the wounded plants in the suspension of *Foc* conidia in INO-2 method guaranteed the initial *Foc* infection. Although tested abaca plants were exposed to mixtures of potting medium with high amount of *Foc* hyphae (INO-1) or conidia (INO-3), INO-1 and INO-3 methods did not guarantee infection because of the absence of wounding of abaca roots. Wounding has been shown to assist fungal penetration into inoculated plant tissues (Yusnita & Sudarsono 2004; Sakamoto & Gordon 2006).

According to stages of *Foc* infection, damaged of abaca root tissues started at the epidermis, followed by cortex and xylem tissues, consecutively. The stages of *Foc* infection were initiated with attachment and penetration in epidermal tissues of abaca roots and followed by spreading and collonization in xylem tissues (Salerno *et al*. 2000; Mak *et al*. 2004a). Observed wilting symptoms of abaca were the results of blocking of xylem tissues by *Foc* mycelia and by induction of thylosis because of activities of secreted enzymes and fusaric acid by *F. oxysporum* (Salerno *et al*. 2000).

Root tissue damage at 30 DAI reached only the cortex tissues. Observation of root histology indicated that damaged root tissues showed dark brown color due to *Foc* infection. The observed brownish color in damaged root tissues were because of phenolic compounds degradation into less simple compounds by phenol oxydase secreted by *Foc* and by absorption of the compounds by cell wall of the damaged root tissues (Semangun 1996). Therefore, changes of the

infected root tissue into brownish or blackish color could indicate the presence of damages in the root tissue of abaca caused by *Foc* inoculation.

Although root damages at 30 DAI reached only the cortex, the tested abaca plants have shown average scores of wilting symptom ranged from 0.3 to 1.7. The cortex consisted of parenchyme cells. The functions of the cells were associated with water transportation and oxygen storage in roots. Cortex role did not directly related to water transportation. However, the cortex damaged might indirectly affect water transportation from root to shoot and leaf tissues and result in wilting symptoms.

At 60 DAI, the percentage of abaca plants showing wilting symptoms and disease intensities due to *Foc* infection were higher than that at 30 DAI. The increased of disease intensity at 60 DAI might probably be the result of damages in root xylem tissues due to *Foc* infection. The xylem was responsible for water and nutrient transportation in plants. The damaged xylem might have direct effects on water transport from roots to leaves; therefore, it might also have direct effects on causing wilting symptoms. However, further study is needed to verify this since root damages at 60 DAI was not observed in this experiment.

For abaca with severe symptoms, *Foc* infection also caused necrotic symptoms at the base of plants. In this research, the abaca 'Tangongon' with severe wilting symptom showed necrotic symptoms at the base of plants of only less than 3.2 and disease intensities of less than 64% in average. This might indicate that *Foc* at 60 DAI might only cause root damage and the damage has only reached cortex tissues of the roots. If the damages have reached the xylem tissues, the tested plants would have been showed score of wilting symptom at score 4 and disease intensity close to 100%.

It is obvious that high density of *Foc* conidia has effectively increased the disease intensity based on the calculation of the both the wilting symptoms and the necrotic symptoms at the base of abaca plants at 60 DAI. According to Agrios (1997), higher densities of inocullum has higher potential for causing disease symptoms than lower ones. Ben-Yephet *et al*. (1996) reported the existance of positive correlation between densities of inocullum and the occurrence of Fusarium wilt on *Dianthus* sp., while Mak *et al*. (2004b) reported that differences in inocullum densities resulted in different responses among tested plants. On *Musa* sp., the use of *Foc* conidia at densities of 5×10^2, 5×10^4, or 5×10^6 conidia/ml resulted in mild, medium, and severe disease incidence, respectively (Mak *et al*. 2004b).

Using developed screening methods, nine of the tested abaca cultivars were identified as very susceptible (Vs) and one was susceptible against *Foc* infection (Sc). Basuki (2003) stated that crops species such as banana (*Musa* sp.) and cotton (*Gossypium* sp.) did not have resistance mechanism against Fusarium wilt. On the other hand, tomato (*Lycopersicon esculentum*) was reported to have a number of resistance genes against infection of *F. oxysporum* f.sp. *lycopersici* (*Fol*). According to Burge *et al*. (2003), *Cucumis melo* has a resistance gene against infection of *Fusarium oxysporum* f.sp. *melonis*.

In Indonesia, it has been reported that genetic diversity of abaca germplasm collections based on molecular marker study was low (Hadipoentyanti *et al*. 2001). Roux *et al*. (1999) stated that vegetatively propagated crops such as *Musa* spp. generally exhibited limited genetic diversities since male is usually sterile and polyploid. Therefore, similar responses of 10 abaca cultivars against *Foc* infection might be due to the absence of resistance gene to *Foc* infection and to the limited genetic diversity of the Indonesian abaca germplasm collections.

Based on the data from this research, it can be concluded that inoculation method by wounding abaca roots followed by dipping wounded abaca plants on suspension of 10^6 *Foc* conidia/ml might be used to screen the responses of abaca germplasms against *Foc*. Based on the value of observed disease intensities, ten abaca cultivars grown in Indonesia were identified as either susceptible or very susceptible against *Foc* infection. Therefore, increasing abaca genetic diversity, especially with resistance to *Foc* infection is needed. Since abaca is one of vegetatively propagated crops, induced mutation or somaclonal variation might be used as alternative methods for increasing abaca genetic diversity. Combining these methods and *in vitro* selection on medium containing culture filtrate (toxin) of *Foc* are expected to be more effective for generating *Foc* resistance abaca than by introducing and evaluating more abaca germplasms from other countries. Using *in vitro* mutation, induction of variant cells or tissues of abaca can be conducted. Culture filtrate (toxin) tolerance mutant could be identified by subjecting the cultures on selecting medium containing culture filtrate (toxin) of *Foc*. Subsequently, regenerated plants from selected mutant tissues should also be tolerance against secreted *Foc* toxin during infection process. In other words, the selected abaca mutant is expected to be *Foc* resistance.

ACKNOWLEDGEMENT

Part of this research was supported by Participatory Assessment of Agriculture Technology Project (PAATP) No. SK: 36.Kp.510/PAATP/02/2001, date: February 12, 2001. The authors would like to sincerely thanks to the Chairman of Agency of Agriculture Research and Development, Central Research Institute of Estate Crops, Central Research of Tobacco and Fiber Crops and the PAATP Project Coordinator for their support to senior author to pursue PhD degree at Bogor Agricultural University and for the provided research facilities; to Edy Guharja and Said Harran for their suggestions and corrections of this manuscript.

REFERENCES

Agrios GN. 1997. *Plant Pathology*. 4th ed. San Diego: Acad Pr, Inc.

Bastasa GN, Baliad AA. 2005. Biological control of Fusarium wilt of abaca (*Fusarium oxysporum*) with *Trichoderma* and yeast. *Philippines J Crops Sci* 30:29-37.

Basuki S. 2003. Identification of DNA markers and recombinations events in the vinicity of the *Fusarium oxysporum* f.sp. *licopersici* resistance gene I-3 of tomato (*Lycopersicon esculentum*) [Thesis]. Brisbane: School of Land & Food Sciences and School of Molecular & Microbiology Sciences, The University of Queensland.

Ben-Yephet Y, Reuven M, Zviebil A, Shtienberg D. 1996. Effects of initial inoculum and cultivar resistance on incidence of Fusarium wilt and population densities of *Fusarium oxysporum* F.sp. *dianthi* on carnation and in soil. *Phytopathology* 86:751-756.

Burge Y *et al*. 2003. Variation in the resistance of melon genotypes to *Fusarium oxysporum* f.sp. *melonis* race 1 determine by inoculation tests and molecular markers. *Plant Pathol* 52:204-211.

Cachinero JM, Hervas A, Jimenez-Diaz RM, Tena M. 2002. Plant defence reactions against Fusarium wilt in chickpea induced by incompatible race 0 of *Fusarium oxysporum* f.sp. *ciceris* and non-host isolates of *F. oxysporum*. *Plant Pathol* 51:765-776.

Di Pietro A, Madrid MP, Caracuel Z, Delgado-Jarana J, Roncero MIG. 2003. *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. *Mol Plant Pathol* 4:315-325.

Djatnika I, Hermanto C, Eliza. 2003. Pengendalian hayati layu Fusarium pada tanaman pisang dengan *Pseudomonas fluorescens* dan *Gliocladium* sp. *J Hort* 13:205-211.

Epp D. 1987. Somaclonal variation in banana: a case study with Fusarium wilt. In: Persley GJ, De Langhe EA (eds). *Banana and Plantain Breeding Strategies*. Canberra: ACIAR Publ. p 140-150.

Gregory PH. 1983. Spore Trapping. In: Johnson A, Booth C (eds) *Plant Pathologist's Pocketbook*. Slough: CMI. p 328-331.

Hadipoentyanti E, Ratnadewi D, Solihat L. 2001. Variabilitas genetik berbagai varietas abaka (*Musa textilis* Nee) dan kerabat liar melalui analisis RAPD. *Zuriat* 12:93-106.

Hwang SC, Ko WH. 2004. Cavendish banana cultivars resistant to Fusarium wilt acquired through somaclonal variation in Taiwan. *Plant Dis* 88:580-588.

Ishak, Dwimahyani I. 2005. Evaluasi pertumbuhan dan ketahanan galur mutan pisang terhadap *F. oxysporum* fsp. *cubense* (FOC). *J Stigma* 13:26-30.

Mak C, Ho YW, Liew KW, Asif JM. 2004a. Biotechnology and *in vitro* mutagenesis for banana improvement. In: Jain SM, Swennen R (eds). *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations.* Enfield: Sci Pub, Inc. p 51-68.

Mak C, Mohamed AA, Liew KW, Ho YW. 2004b. Early screening technique for Fusarium wilt resistance in banana micropropagated plants. In: Jain SM, Swennen R (eds). *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations.* Enfield: Sci Publ, Inc. p 11-20.

Nasir N, Jumjunidang. 2003. Karakterisasi ras *Fusarium oxysporum f.sp. cubense* dengan metode *vegetative compatibility group test* dan identifikasi kultivar pisang yang terserang. *J Hort* 13:276-284.

Nasir N, Jumjunidang, Eliesti F, dab Meldia Y. 2003. Penyakit layu panama pada pisang: observasi ras 4 *Fusarium oxysporum* f.sp. *cubense* di Jawa Barat. *J Hort* 13:269-275.

Roux N *et al*. 1999. Usefulness of embriogenic cell suspension for the induction and selection of mutants in *Musa* spp. *Promusa* 4:18-19.

Sakamoto JM, Gordon TR. 2006. Factors influencing infection of mechanical wounds by *Fusarium cincinatum* on Monterey pines (*Pinus radiata*). *Plant Pathol* 55:130-136.

Salerno MI, Gianinazzi S, Gianinazzi-Pearson V. 2000. Effects on growth and comparison of root tissue colonization pattern of *Eucaliptus viminalis* by pathogenic and non-pathogenic strains of *Fusarium oxysporum. Pythol* 146:317-324.

Semangun H. 1996. *Pengantar Ilmu Penyakit Tumbuhan*. Ed ke-3. Yogyakarta: Universitas Gadjah Mada Pr.

Setyo-Budi U, Sudjindro, Purwati RD. 2001. Status plasma nutfah abaka: menyongsong agribisnis abaka. In: Kasno A *et al.* (eds). *Kontribusi Pemuliaan dalam Inovasi Teknologi Ramah Lingkungan*. Bandung: Perhimpunan Ilmu Pemuliaan Indonesia (PERIPI). p 254-258.

Yusnita, Sudarsono. 2004. Metode inokulasi dan reaksi ketahanan 30 genotipe kacang tanah terhadap penyakit busuk batang Sclerotium. *Hayati* 11:53-58.