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Research Article

Race and Virulence Determination of *Fusarium oxysporum* f. sp. *cubense* Isolates from Sidomulyo Village of Bantul, Yogyakarta

Penentuan Ras dan Virulensi Isolat Fusarium oxysporum *f. sp.* cubense *Asal Desa Sidomulyo Kabupaten Bantul*

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ABSTRACT

Banana is one of the important fruit crop in Village of Sidomulyo, Bantul, Yogyakarta. One of important diseases which become the constraint in development of banana is Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). This fungus has high race diversity and virulence, so that it required early detection for prevention and control of disease. This experiment was aimed to figure out race and virulence of *Foc* isolates from Village of Sidomulyo, Bantul, Yogyakarta. The 13 tested isolates were isolates of PR11, PKJ20, RU20, PR30, AH40, PKJ40, A41, RB42, PR43, RU51, A60, RP60, and A80. Race was molecularly detected using two types of primers, i.e. General *Foc* primer FocEf3 and specific primer for race 4 (Foc-1/Foc-2). Virulence test was performed on banana seedlings of Ambon Kuning cultivar using Completely Randomized Design (CRD) with 14 treatments and 4 repetitons. The observed parameters were external and internal symptoms, calculation of disease severity index and disease intensity. Data were analyzed using variance and further test of Duncan Multiple Range Test (DMRT) at 5 % level. The results showed that all isolates were *Foc* and 9 of 13 isolates were grouped into race 4, i.e. A80, RP60, PR11, A41, AH40, PKJ40, PR30, RB42, and PR43. The highest and lowest virulences were consecutively expressed by PR30, RB42, RU51, RP60, PR43, PKJ40, PR11, A41, AH40, RU20, PKJ20, A60, and A80, with severity index on leaves and rhizomes ranging 1.61−2.91 and 2.25−7, respectively.

Keywords: banana, *Fusarium oxysporum* f. sp. *cubense*, race, virulence

INTISARI

Pisang merupakan tanaman buah unggulan di Desa Sidomulyo Kecamatan Bambanglipuro, Kabupaten Bantul. Salah satu penyakit penting yang menjadi kendala dalam pengembangan pisang adalah layu fusarium yang disebabkan oleh jamur Fusarium oxysporum *f. sp.* cubense *(*Foc*). Jamur ini memiliki keragaman ras dan virulensi yang tinggi, sehingga deteksi dini diperlukan untuk pencegahan dan pengendalian penyakit. Penelitian ini bertujuan untuk mengetahui ras dan virulensi isolat* Foc *asal Desa Sidomulyo, Kecamatan Bambanglipuro, Kabupaten Bantul. Isolat yang diuji sebanyak 13 isolat, yakni isolat PR11, PKJ20, RU20, PR30, AH40, PKJ40, A41, RB42, PR43, RU51, A60, RP60, dan A80. Pengujian ras secara molekuler dengan menggunakan dua jenis primer yakni primer* Foc in general *FocEf3 dan primer spesifik ras 4 Foc-1/Foc-2. Uji virulensi pada bibit kultivar ambon kuning dengan menggunakan Racangan Acak Lengkap (RAL) yang terdiri dari 14 perlakuan dan 4 ulangan. Parameter yang diamati berupa pengamatan gejala luar dan gejala dalam, penghitungan indeks keparahan penyakit dan intensitas penyakit. Analisis data menggunakan sidik ragam dan uji lanjut* Duncan Multiple Range Test *(DMRT) pada taraf 5 %. Hasil pengujian menunjukkan bahwa semua isolat merupakan isolat* Foc *dan dari 13 isolat yang digunakan terdapat 9 isolat yang merupakan ras 4 yakni isolat A80, RP60, PR11, A41, AH40, PKJ40, PR30, RB42, dan PR43. Isolat yang memiliki virulensi tertinggi sampai terendah berturut-turut adalah PR30, RB42, RU51, RP60, PR43, PKJ40, PR11, A41, AH40, RU20, PKJ20, A60, dan A80, dengan indeks keparahan pada daun berkisar 1,61−2,91 dan indeks keparahan pada bonggol 2,25−7.*

Kata kunci: Fusarium oxysporum *f. sp.* cubense*, pisang, ras, virulensi*

INTRODUCTION

Banana is one of important economic horticultural crops which was cultivated by farmers in Village of Sidomulyo, Bantul, Yogyakarta. Data of fruit crops in this village placed it as the most planted one, i.e. about 15,957 plants, followed by 2,706 plants of mango, 2,288 plants of melinjo and 1,754 plants of rambutan (BKP3, 2016). According to data of BPS (2016), banana production in Village of Sidomulyo was equivalent to total of productions in two other villages within District of Bambanglipuro, namely 200.9 ton in Sidomulyo, 100.5 ton in Mulyodadi and 100.4 ton in Sumbermulyo.

The development of banana plantation can not be separated from the prevalence of crop disease. One of important disease invading banana crop is Fusarium wilt caused by *Fusarium oxysporum* Schlecht f. sp. *cubense (Foc)* (E.F. Smith) Snyder & Hansen (foc) (Ploetz, 2006; Ghag *et al*., 2015). Diseased plant will express the symptoms of yellowing leaves from the lower ones, wilt and longitudinal section of rhizomes showed the brown blackish stripe towards up all parts of plant through pseudostem (Semangun, 2000). This disease is very dangerous and threatening banana industry in the world (Moore *et al*., 1995; Visser, 2010). It has been reported destroying more than 40,000 ha of banana orchards in Central America an South America for period of 50 years (Su *et al*., 1986). In Indonesia, the infection has been detected in whole regions from Aceh to Papua (Hermanto *et al.*, 2011; Jumjunidang *et al*., 2012) and abolish thousands hectares of commercial and private banana plantations (Nasir *et al*., 2005).

Foc establishes four races based on its pathogenicity against several banana cultivars. Race 1 infects banana cultivars of Gros Michel (AAA), 'Maqueno' (Maia Maoli-Popoulu subgroup, AAB), Silk, Pome, and Pisang Awak (ABB). Race 2 is recognized affecting cultivar of Bluggoe (ABB). Race 3 attacks group of *Heliconia* spp. Race 4 is known as the most dangerous infecting Cavendish as well as race 1 and 2-susceptible cultivars. Race 4 is categorized into 2 types, namely subtropical race 4 (SR4) and tropical race 4 (TR4) (Ploetz. 2015). In Indonesia, this race has been dispersed in some banana producing areas in Java, Lampung, and Kalimantan (Wibowo *et al*., 2007).

The high diversity in race and virulence of *Foc* enabled early detection is required very much in efforts of prevention and controlling of Fusarium wilt disease. Molecular identification from various isolates can be referred as basic of race categorization on *Foc* (Kuswinanti *et al*., 2011). This research was aimed to figure out race and virulence of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) isolates from Village of Sidomulyo, Bantul, Yogyakarta.

MATERIALS AND METHODS

Molecular Identification on Race of Fusarium oxysporum *f. sp.* cubense *Isolates*

Sample collection. Samples were randomly collected from 7 subvillage in Village of Sidomulyo, District of Bambanglipuro, Regency of Bantul, namely Ponggok, Pinggir, Plebengan, Plemantung, Sirat, Selo, and Cangkring. One infected cultivar was considered as one sample, whereas if more diseased cultivars were found, the samples were taken from each cultivar.

Sampled banana plants showing fusarium wilt symptoms were cut by chopping the pseudo-stem about 20−30 cm from neck of rhizome with approximately 5×15 cm in size. The internal part of infected pseudo-stem expressed reddish or brownish color. Afterwards, the vessel spindles were gently pull out to separate them from tisses, air-dried, put on filter paper, covered with steril tissue paper, kept into envelopes and then put into closed plastic box containing silica gel. The number of obtained samples were 13 as shown in Table 1.

Isolation and purification of **Fusarium oxysporum *f. sp.* cubense *isolates.*** The air-dried tissues of pseudostem were cut about 0.5–1 cm, cultured on Potato Dextrose Agar (PDA) medium containing 1 drop of 25% lactic acid and then incubated under room temperature for 7 days (25ºC–27ºC). The isolated fungi were then purified using single spore isolation technique. This technique was performed by diluting the fungal colony with 10 ml of sterile aquadest and vortexing for 2 min. Furthermore, fungal suspension was streaked onto water agar (WA) medium using ose needle, incubated for 15 h at room temperature (25ºC–27ºC) , and then the single germinating spore was transferred onto PDA medium and incubated for 7 days at room temperature (25ºC–27ºC).

Table 1. The used samples in this experiment

No.	Code of Sample	Origin Sub-Village	Infected Cultivars
1	PR11	Ponggok	Pisang Raja Bagus (AAB)
2	PKJ20	Pinggir	Pisang Koja / Susu (AAA)
3	RU20	Pinggir	Raja Uter (AAB)
4	PR30	Plebengan	Pisang Raja Bagus (AAB)
5	AH40	Plemantung	Ambon Hijau (AAA)
6	PKJ40	Plemantung	Pisang Koja / Susu (AAA)
7	A41	Plemantung	Ambon Kuning (AAA)
8	RB42	Plemantung	Raja Bulu (AAB)
9	PR43	Plemantung	Pisang Raja Bagus (AAB)
10	RU51	Sirat	Raja Uter (AAB)
11	A60	Selo	Ambon Kuning (AAA)
12	RP60	Selo	Raja Pulut (AAB)
13	A80	Cangkring	Ambon Kuning (AAA)

Table 2. PCR program using primers of *Foc in general* FocEf3 (Widinugraheni *et al*., 2015)

Temperature,ºC	Time	Stage	Cycle	Amplified Target
94	5 min	Pra-denaturation	1	
94	30 s	Denaturation		
57	30 s	Annealing	30 cycles	600 bp
72	2 min 30 s	Extension		
72	5 min	Final Extension	1	

Table 3. PCR program using specific primer for race 4 of Foc-1/Foc-2 (Lin *et al*., 2008)

Temperature,ºC	Time, min	Stage	Cycle	Amplified Target
95	5	Pra-denaturation	1	
95	1	Denaturation		
55	1	Annealing	33 cycles	242 bp
72	3	Extension		
72	10	Final Extension	1	

DNA isolation from isolates of* Fusarium oxysporum *f. sp. cubense. Fungi were cultured in 40 ml of Potato Dextrose Broth (PDB) medium in Erlenmeyer and stirred using shaker for 1 week at room temperature. DNA was extracted using CTAB method of Subandiyah (2003).

DNA amplification. DNA amplification was carried out using two primers, i.e. primer of *Foc in general* FocEf3 (Widinugraheni, 2015) to ensure that all tested isolates were *Fusarium oxysporum* f. sp. *cubense* and specific primers for race 4 of Foc-1/Foc-2 (Lin *et al*., 2009). PCR program for those primers were shown in Table 2 and Table 3, respectively.

Detection of Fragments by Electrophoresis

The next stage was electrophoresis on PCR product using 1 % of agarose gel in TBE 1x solution. As much of 5 µl PCR product was pipetted into well of agarose gel as well as 5 µl of 100 bp marker to indicate the size of DNA bands. Electrophoresis was run for 45 min at 50 volt. Gel was stained by dipping in ethidium bromide (EtBr) solution for 15 min and then rinsed with sterile aquadest. DNA bands were visualized and documented on UV transluminator.

Virulence Assay of* Fusarium oxysporum *f. sp.* cubense *Isolates on Banana Seedling

Preparation of Inoculum

Fusarium oxysporum f. sp. *cubense* isolates were culture on slant agar in reaction tubes containing PDA medium and incubated for 7 days. Conidia suspension was prepared by adding 10 ml of sterile aquadest into test tubes and harvesting the mycelia using ose needle. Afterwards, conidia concentration was adjusted to 10^7 conidia/ml water.

Inoculation of Foc on Banana Seedling of Ambon Kuning

This experiment was completely randomized design (CRD) with 14 treatments and 4 repetitions. The used banana plants were 6-month tissue culture seedlings of Ambon Kuning cultivar obtained from Banana Germplasm Orchard of Yogyakarta. The seedlings were planted in polybag containing sterile soil. Artificial inoculation was performed by injuring the rooting and rhizome areas using sterile scalpel and then pouring the conidia suspension into those areas. Control used sterile water. The inoculated seedlings were kept in the glass house.

Virulence Assay

Observation on external symptom. The observation was weekly conducted for 7 weeks. The observed parameter was wilt symptom on leaf, and then analyzed according to leaf symptom index (LSI) using method of Mak *et al*. (2004) which had been modified by Kiswanti *et al.* (2010) as displayed in Table 4.

Observation on internal symptoms. The observation of rotting symptom on rhizome (as known as Rhizome Discoloration Index/RDI) was carried out at 7th week after inoculation, and then analyzed according to method of Mak *et al*. (2004) which had been modified by Kiswanti *et al.* (2010) as shown in Table 5.

Table 4. Leaf Symptom Index (LSI)

Scoring	Remarks
0	No wilting symptom/healthy plant
1	1−2 yellowing/wilting leaves
2	3−4 yellowing/wilting leaves
3	5 yellowing/wilting leaves
4	>5 yellowing/wilting leaves

Calculation of Disease Severity Index

Disease Severity Index (DSI) was overall counted based on RDI and LSI data using following formulation:

$$DSI = \frac{\Sigma\,(score\ x\ number\ of\ corresponding\ plant)}{\Sigma\,(number\ of\ tested\ plants)}$$

Virulence of isolates was determined by results of LSI and RDI following method of Mak *et al*. (2004) which has been modified by Kiswanti *et al*. (2010) as shown in Table 6.

Observation of Disease Development

Disease development was observed according to number of yellowing leaves on one plant (Wibowo *et al*., 2001). Scoring for observation of yellowing leaves on banana plants was based on the following Table 7 (Sumardiyono, 2001).

Having leaf observation, the disease intensity was calculated with formulation as below:

$$IP = \frac{\sum_{i=0}^{Z}(ni\ x\ vi)}{N\ X\ Z}\ \text{x}\ 100\%$$

Note:

IP : Disease Intensity

ni : Number of leaves on each corresponding score

vi : Score of disease on corresponding leaf

Z : Highest score

N : Number of observed leaves

Data Analysis

Disease intensity from first week until the 7th week were analyzed using ANOVA. If there is significantly different, it was further analysis using Duncan Multiple Range Test (DMRT) at 5 % level.

Table 5. Rhizome Discoloration Index (RDI)

Scoring	Remarks
0	No discoloration/rotting on rhizome and rooting area or surrounding tissue
1	No discoloration/rotting on rooting area, discoloration found on root branches only
2	Discoloration/rotting up to 5% on rhizome
3	Discoloration/rotting up to 6-20% on rhizome
4	Discoloration/rotting up to 21-50% on rhizome
5	Discoloration/rotting up to >50% on rhizome
6	Rotting on whole parts of rhizome and rooting area
7	Plant died

Table 6. Remarks on DSI scale

Scale of DSI for LSI	Scale of DSI for RDI	Remarks
0	0	Avirulent
0.1 – 1.0	0 – 2.0	Moderate
1.1 – 2.0	2.1 – 4.0	Virulent
2.1 – 3.0	4.1 – 7.0	High virulent

Table 7. Scoring grade of yellowing leaves for observation of disease intensity

Scoring	Precentage of disease severity
0	Healthy leaves
1	1 yellowing/wilting leaf
2	2−3 yellowing/wilting leaves
3	4−5 yellowing/wilting leaves
4	>5 yellowing/wilting leaves

RESULTS AND DISCUSSION

Molecular Identification on Race of Foc

The result showed that all isolates could be amplified with primers of *Foc in general* FocEf3 which was indicated by the presence of DNA bands at 600 bp in size (Figure 1a). It could be concluded that all tested isolates were *Fusarium oxysporum* f. sp. *cubense.*

The electrophoresis of PCR product using specific primers for race 4 of Foc-1/Foc-2 expressed that 9 of 13 isolates could be amplified at 242 bp in size (Figure 1b). Primer of Foc-1/Foc-2 had high specificity in detecting the isolates of *Foc* race 4 (*Foc* subtropical race 4 (SR4) and tropical race 4 (TR4) (Lin *et al*., 2009). However, isolates of PKJ20, A60, RU20 and RU51 were not amplified using those primers. Those isolates were assumed to be categorized into *Foc* race 1 since the infected hosts were cultivars of pisang Koja/pisang susu (AAA), pisang ambon kuning (AAA) and pisang raja uter (AAB). Jeger *et al*. (1995) reported that *Foc* race 1 was pathogenic against banana cultivar having genomes of AAA and AAB.

Virulence Assay on Isolates of Foc *on Banana Seedling of Ambon Kuning*

Disease severity could be considered as one of foundation in determining the virulence level of pathogen. It was measured as percentage of infected part of plants such as roots, leaves or stems with corresponding symptoms generated by given pathogen (Pariaud *et al*., 2009). The level of disease severity using isolates of *Foc* on banana seedlings in this experiment could be seen by observating external symptoms (yellowing or wilting leaves) and internal symptoms (rotting of rhizome). This research documented LSI in range of 0.18–3.13 and RDI of 0–7 in range as expressed in Table 8.

Analysis of DSI against using LSI and RDI data showed that 8 of 13 isolates were very virulent, i.e. isolates of PR11, A41, AH40, PKJ40, RU51, PR30, RB42, and PR43; while other isolates (PKJ20, A80, RP60, A60, and RU20) were virulent. The difference in virulence occurred because of the difference in biological, chemical, genetic and ability of asexual spore reproduction on each isolate (Groenewald, 2005; Jumjunidang *et al*., 2011). All of high virulent isolates were grouped into race 4 excluding isolate of RU51. Su *et al.* (1986) and Ploetz (2006) explained that *Foc* race 4 was the most terrible and virulent race since it was able to infect all types of banana including those which were susceptible against race 1 and 2.

The difference in categorization of disease severity level could also be viewed from LSI and RDI data on isolate of RU20 (Table 8). LSI data of this isolate indicated that it belonged to high virulent category, while RDI data revealed that isolate of RU20 was virulent. However, the categorization of virulence referred to RDI data since the infection of pathogen initiated from rooting and rhizome areas. According to Ploetz (2000), *Foc* penetrated to plant tissues through root and then developed towards stem and extend to vessel tissue. Fungal development in tissue affected the interruption of water and nutrient flow from soil so that the plants was getting wilt which was indicated by the yellowing of bottom leaves. The control plant expressed LSI data about 0.18, which was obtained from the number of yellowing leaves during observation. However, these yellowing leaves were assumed due to the aging stage of leaves. It was proved by RDI data which did not find the rotting symptoms either on rhizome or rooting areas.

Disease Progress

The results showed that the symptom of Fusarium wilt disease on banana seedling of ambon kuning emerged on 1st week after inoculation, excluding for

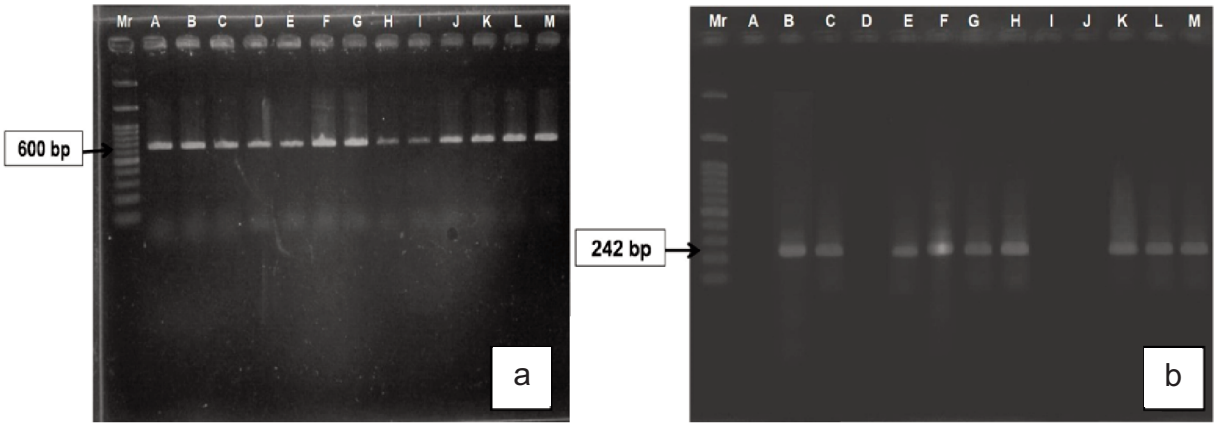

Figure 1. Results of electrophoresis from amplification using primer of *Foc in general* FocEf3 (a), and specific primer for race 4 of Foc-1 and Foc-2 (b); Marker 100 bp (Mr), PKJ20 (A), A80 (B), RP60 (C), A60 (D), PR11 (E), A41 (F), AH40 (G), PKJ40 (H), RU20 (I), RU51 (J), PR30 (K), RB 42 (L), PR43 (M)

Table 8. DSI analysis on isolates of *Foc* on banana seedling of Ambon Kuning

No.	Code of Isolates	DSI based on LSI	DSI based on RDI	Remarks
1	PKJ20	1.75	3	Virulent
2	A80	1.61	2.25	Virulent
3	RP60	1.64	4	Virulent
4	A60	1.89	3.75	Virulent
5	PR11	2.59	5.5	High Virulent
6	A41	2.56	5	High Virulent
7	AH40	2.28	5	High Virulent
8	PKJ40	2.69	4.75	High Virulent
9	RU20	2.14	3.75	Virulent
10	RU51	2.91	5.25	High Virulent
11	PR30	2.88	7	High Virulent
12	RB42	3.13	6.25	High Virulent
13	PR43	2.63	6	High Virulent
14	Control	0.18	0	-

control (Figure 2). This early emergence of symptom might be caused by pre-injuring on plant rooting and high concentration of applied inoculum. According to Agrios (2005), the wounding of plant would enable the pathogen to penetrate and introduce into plant tissue. During infection, pathogen would grow, reproduce and colonize the plant. The successful infection process would generate the symptom on plant.

The used inoculum density in this experiment could be considered as high concentration, i.e. 10^7 conidium/ml water. Mak *et al*. (2004) reported that conidium density of *Foc* with concentration of 5×10^6 conidium/ml water had been able to appear the symptom at 10th day after inoculation. Therefore, the chance and ability of pathogen to infect with the higher concentration (10^7 conidium/ml water) were increasing so that the emergence of symptom would be earlier. It was paralel to Agrios (2005) and Riska *et al.* (2012) who explained that high inoculum density would fasten disease development, improve the ability to generate symptom, and increase disease severity.

It was found the difference in development of disease intensity corresponding to each isolate of *Foc* until 7th week observation. Disease intensity started to quickly increase at 3rd week after inoculation. At the last week after inoculation, it could reach about 58.71% to 97.73%. The plant which was inoculated with isolate of PR30 expressed the disease intensity about 26.01% at 3rd week and up to 97.73% at 7th week. Meanwhile, disease intensities caused by isolate of A80 were about 8.8% and 58.71% at 3rd and 7th week, respectively.

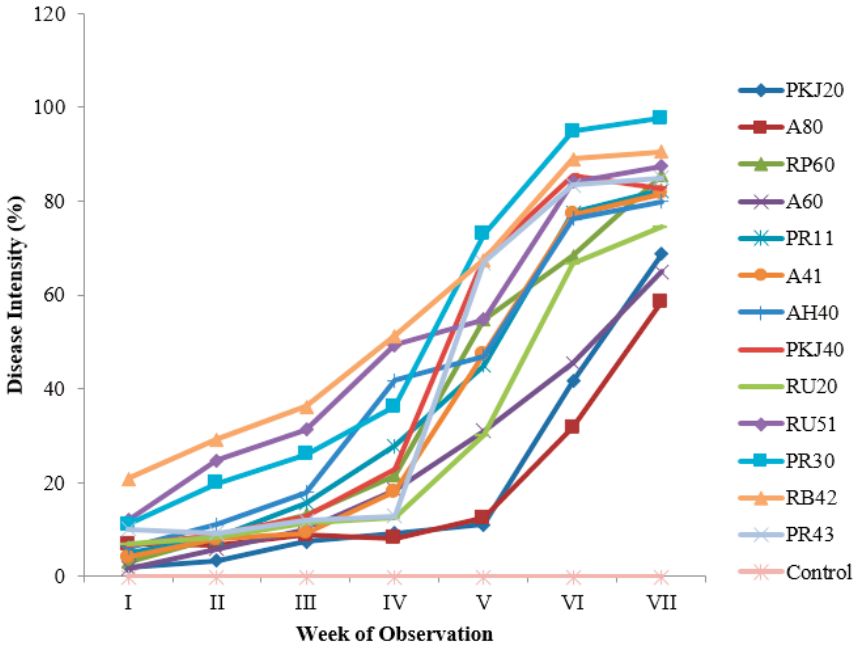

Figure 2. Development on intensity of fusarium wilt disease on banana of Ambon Kuning cultivar artificially inoculated with 13 isolates of *Foc* and 1 control from 1st to 7th week after inoculation

Means of disease intensity at 7th week after inoculation showed that the highest disease intensity was found on treatment with isolate of PR30 about 97.73%, followed by isolates of RB42, RU51, RP60, PR43, PKJ40, PR11, A41, AH40, RU20, PKJ20, A60, A80, and control about 90.87%; 87.50%; 85.49%; 84.98%; 82.52%; 82.35%; 81.59%;79.79%; 74.46%; 68.75%; 64.94%; 58.71%; and 0%, respectively. It could be concluded that isolate of PR30 had highest virulence level and isolate of A80 was the lowest (Table 9).

The variance analysis in Table 9 indicated that treatment with PR30 was not significantly different to treatments with isolates of RB42, RU51, RP60, PR43, PKJ40, PR11, A41, AH40, and RU20, but it was significantly different to treatments with isolates of PKJ20, A60 and A80.

The development of disease intensity was supposed to be related to factors of pathoen and plant. Pathogen in this case was correlated to high density of inoculated inoculum, so that it had high capability to cause the disease. According to Purwanti *et al.* (2008), high density of *Foc* conidia was effective in raising disease intensity based on the symptom of yellowing and wilting leaves on Abaca plant. In addition, the virulence of pathogen also influenced disease intensity. The highest disease intensity was found on isolate of PR30 which was race 4 of *Foc*. It was recognized having high virulence in attacking all banana cultivars. High disease intensity was also presented by isolate of RU51 which was considered as race 1 of *Foc*. Previously, Bentley *et al.* (1998) explained that race 1 of *Foc* could affect Gros Michel cultivar and even destroy banana industry in the world. Recently, Hermanto *et al.* (2013) reported that pisang ambon kuning belonged to Gros Michel group which might be infected with high percentage as well.

Table 9. Means of disease intensity on banana plant of Ambon Kuning inoculated with 13 isolates of Foc and 1 control at 7th week after inoculation

Isolates	Disease Intensity (%)
PR30	97.73 a
RB42	90.87 ab
RU51	87.50 abc
RP60	85.49 abc
PR43	84.98 abc
PKJ40	82.52 abc
PR11	82.35 abc
A41	81.59 abc
AH40	79.79 abc
RU20	74.46 abcd
PKJ20	68.75 bcd
A60	64.94 cd
A80	58.71 d
Control	0 e

Remark: Numbers followed by dissimilar letter were not sigificantly different at 5 % level according to DMRT (arcsin transformation)

The ability in producing toxin of fusaric acid also affected the virulence of each tested *Foc* isolate. The higher production of fusaric acid, the higher virulence and disease intensity of isolates. It was indicated by the development of wilting or yellowing leaves (Dong *et al*., 2012). Fusaric acid was a phytotoxin produced by *Foc* which disrupted the permeability of plant membrane, inhibited the oxygen taking and had important role as causal agent of yellowing leaves and enhanced the aging process on infected plants (Dong *et al*., 2014).

Susceptible plant and less nutrient soil could improve the emergence of disease when they were inoculated with virulent pathogen. This experiment used cultivar of ambon kuning which was one of susceptible cultivars against *Foc* and the plants were not treated with additional nutrients so that they did not sturdy grow and were getting susceptible against *Foc*.

CONCLUSION

This investigation concluded that all tested isolates were *Foc* and 9 of 13 isolates were categorized into race 4, i.e. A80, RP60, PR11, A41, AH40, PKJ40, PR30, RB42, and PR43. All isolates could be successively listed from the most to the least virulent, i.e. PR30, RB42, RU51, RP60, PR43, PKJ40, PR11, A41, AH40, RU20, PKJ20, A60, and A80, with LSI ranged 1.61−2.91 and RSI was about 2.25−7. High virulence and abundance of race 4 from obtained isolates of Sidomulyo Village are expected to increase the early awareness of farmers in cultivating banana plant. The use of healthy and non-susceptible banana seedlings in *Foc*-infected soils was suggested to farmers in Village of Sidomulyo, District of Bambanglipuro, Regency of Bantul.

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