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Resistance Responses of 35 Watermelon Genotypes to Three Isolates of *Fusarium oxysporum* f. sp. *niveum*

(Respons Ketahanan 35 Genotipe Semangka terhadap Tiga Isolat *Fusarium oxysporum* f. sp. *niveum*)

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ABSTRAK

Layu Fusarium yang disebabkan oleh cendawan *Fusarium oxysporum* f. sp. *niveum* (*Fon*) merupakan salah satu penyakit penting tanaman semangka. Studi yang mengevaluasi ketahanan genotipe semangka terhadap lebih dari satu isolat *Fon* masih sangat terbatas di Indonesia. Penelitian ini bertujuan menguji ketahanan genotipe semangka terhadap tiga isolat *Fon* dari tiga lokasi berbeda di Indonesia. Penelitian menggunakan 35 genotipe semangka dan tiga isolat *Fon* asal Karawang (FK), Lampung (FL), dan Purwakarta (FP). Penelitian menggunakan Rancangan Acak Lengkap dengan dua ulangan. Data indeks penyakit memperlihatkan sebanyak enam genotipe, yaitu New Hope, Sky Mountain, Southern Light, Super Sweet 66, Uranus, dan Yellow Baby, memiliki fenotipe ketahanan moderat sampai dengan tahan terhadap ketiga isolat *Fon* yang diuji. Data masa inkubasi dan persentase tanaman bergejala juga menunjukkan respons yang berbeda antargenotipe, baik terhadap isolat yang sama maupun isolat yang berbeda. Genotipe New Orchid, sebagai contoh, menunjukkan sebanyak 57,14% tanaman uji bergejala dalam waktu kurang dari 10 hari setelah inokulasi (HSI) ketika diuji dengan isolat FL. Di pihak lain, genotipe New Dragon yang diinokulasi dengan isolat yang sama menunjukkan hanya 6,67% tanaman uji yang bergejala pada masa inkubasi lebih dari 23 HSI. Hasil ini mengindikasikan bahwa genotipe semangka yang tahan terhadap semua isolat dapat digunakan pada program pemuliaan untuk merakit galur semangka dengan spektrum ketahanan yang lebih luas terhadap berbagai isolat *Fon*. Genotipe tahan terpilih harus juga memiliki keragaan agronomi yang bagus dan produktivitas tinggi untuk dipertimbangkan sebagai varietas baru semangka.

Kata kunci: Semangka, layu Fusarium, genotipe, ketahanan, gejala penyakit.

ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (*Fon*) is one of main diseases of watermelon. There have been very limited studies that tested watermelon genotypes to more than one isolates of *Fon* in Indonesia. This research aimed to determine the resistance of 35 watermelon genotypes to three *Fon* isolates taken from three different areas in Indonesia. Incubation period (IP) and disease index (DI) of the 35 watermelon genotypes were determined against three *Fon* isolates collected from Karawang (FK), Lampung (FL), and Purwakarta (FP). The experiment was arranged using a Completely Randomized Design with two replications. DI showed that six watermelon genotypes, i.e. New Hope, Sky Mountain, Southern Light, Super Sweet 66, Uranus, and Yellow Baby demonstrated moderate resistance to resistance phenotypes to all tested *Fon* isolates. IP and percentage of symptomatic plants (PSP) showed different responses among genotypes either to the same or to different isolates. Genotype New Orchid, for example, showed 57.14% symptomatic plants in less than 10 days after inoculation (DAI) when tested with FL isolate. Meanwhile, when tested with the same isolate, genotype New Dragon showed only 6.67% symptomatic plants in more than 23 DAI. The result of this study indicated that watermelon genotypes showing resistant to all tested isolates should be useful for breeding program to develop watermelon lines with broader resistance spectrum against *Fon* pathogen. The resistance genotypes selected should also demonstrate good agronomic performances and high yield to be considered as a new watermelon variety.

Keywords: Watermelon, Fusarium wilt, genotype, resistance, disease symptom.

INTRODUCTION

Watermelon (*Citrullus lanatus* var. *lanatus* [Thunb.] Matsum & Nakai) is one of highly consumed fresh fruit with cultivation area as much 6.8% of worldwide cultivation area (Gusmini and Wehner 2005). Watermelon is also among most annual fruit crops cultivated in Indonesia reaching an export value up to 111,275 USD in 2018. However, watermelon production in 2018 decreased by as much as 3.54% compared to that of 2017 (Statistics Indonesia 2018).

One problem of watermelon production worldwide is insect and disease attacks. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (*Fon*) is one of the most destructive diseases which lead to great loss in watermelon production around the world including Indonesia (Egel and Hoke 2007; Dau et al. 2009; Zhou et al. 2010; Budiastuti et al. 2012; Tran-Nguyen et al. 2013; Martyn 2014). This pathogen infects all stages of plants causing damping-off in seedlings or wilt and smaller fruit size in mature plants which eventually causing the plant collapse and die. Fusarium wilt disease is characterized by the brown to reddish discoloration in dissected root crown of the attacked plants (Egel and Martyn 2007; Roberts et al. 2019).

Fon is categorized as a soil-borne disease that is not easily managed (Lü et al. 2014) since it forms infectious chlamydospore to survive for a very long period in the soil (Wechter et al. 2012). Hazardous methyl bromide is often used for watermelon land preparation to anticipate this problem (Everts and Himmelstein 2015). Considering the facts, the best management practice to control this disease is by utilizing resistant cultivars for planting materials (Meru and McGregor 2016).

Fusarium wilt disease development of watermelon has not much been reported in Indonesia. Only few studies have been done related to *Fon* disease development such as that reported by Budiastuti et al. (2012). Identification of *Fon* race has not been reported and there is no study has yet been reported using differential varieties to test watermelon genotypes against Fusarium wilt pathogen in Indonesia. There have been only limited studies related to watermelon breeding that have been reported in Indonesia. These included study on watermelon agronomic characterization (Yasinda et al. 2015; Makful et al. 2019), character heritability prediction (Abdurrahman et al. 2018), and yield potential analysis (Pamuji et al. 2017).

On the other hand, there are various introduced and local watermelon varieties that have been cultivated by Indonesian farmers. Each of the genotypes has different superior characters which make the genotypes are potentially used as parents in watermelon breeding programs. Breeding toward developing watermelon varieties resistance to Fusarium wilt is of great interest to control the disease in cheap and environmentally friendly manners. The objectives of this study were to determine the resistance of 35 watermelon genotypes against three *Fon* isolates taken from three different areas in Indonesia.

MATERIALS AND METHODS

This study was done in Faculty of Agriculture, IPB University, Dramaga District, Bogor, West Java from December 2008 to September 2009. Isolation, purification, identification, and preparation of inoculum substrates for *Fon* isolates were carried out at the Mycology Laboratory of the Department of Plant Protection. The sterilization of the growing media was carried out at the Soil Biology Laboratory of the Department of Soil Science. Evaluation of resistance to the three *Fon* isolates was carried out in the Leuwikopo Greenhouse of the Department of Agronomy and Horticulture.

Plant Materials

Genetic materials used were 35 watermelon genotypes consisted of 31 genotypes that were introduced plants originated from abroad and 4 genotypes were local varieties (Table 1).

Fon Isolation

Plant materials having true Fusarium wilt disease symptoms were sampled from watermelon cultivation fields (Figure 1) in three watermelon production areas, i.e. Central Lampung Regency, Lampung Province (FL isolate), Karawang Regency, West Java Province (FK isolate), and Purwakarta Regency, West Java Province (FP isolate). The symptomatic crowns (lowest part of main stem nearest to the root) having brown circle on its xylem were dissected into tiny cuts (approx. 5 mm × 5 mm). The explants were then rewashed with sterilized water, dried with tissue paper, and subsequently plated onto potato dextrose agar (PDA) media. The isolate cultures were then purified and maintained on PDA until the cultures in Petri dishes demonstrated homogeneity in physical appearance, i.e. colony color, colony form, etc., to obtain pure

Table 1. Watermelon genotypes used in this study.

No.	Genotype	Genetic background*
1.	6372	Local variety (IPB University collection)
2.	Amor	F_1 hybrid (PT East West Indonesia)
3.	Bangkok Flower	F_1 hybrid (Chia Thai, Thailand)
4.	Black Jumbo	Introduction line (Syngenta Seeds, USA)
5.	Charleston Gray	Introduction line (USDA, USA)
6.	Diana Bangkok Dragon	F_1 hybrid (Chia Thai, Thailand)
7.	Dragon Giant 145	Introduction line
8.	Falcon	F_1 hybrid (Chia Thai, Thailand)
9.	Full Orchid	Introduction line (Chung Shin Seed, Taiwan)
10.	Golden Bright	Introduction line (Chung Shin Seed, Taiwan)
11.	Grace	Introduction line (Widegrow, Taiwan)
12.	Hitam Manis	F_1 hybrid 134-6 (F) × 386-2 (M) (Known You Seed, Taiwan)
13.	Jalur Madu	F_1 hybrid (Known You Seed, Taiwan)
14.	King Dragon	Introduction line (Takii Seed, Japan)
15.	Little Boy	Introduction line (Known You Seed, Taiwan)
16.	Mas Kuning	Introduction line (Known You Seed, Taiwan)
17.	Milano	F_1 hybrid WM 2015 × WM 2012 (PT Benih Citra Asia)
18.	New Dragon	F_1 hybrid F28-1-2 (F) × F613 (M) (Known You Seed, Taiwan)
19.	New Hope	F_1 hybrid yellow diploid INR045 (F) from INRA, France × diploid YX0789 (M) from Green Seeds Co. Ltd. (Technisem Asia Co. Ltd., Vietnam)
20.	New Orchid	Introduction line (Known You Seed, Taiwan)
21.	Nina	F_1 hybrid WH 92-357-65 (F) × WH 92-146-81 (M) (Nunhems Zaden BV, Netherlands)
22.	Sky Mountain	Introduction line (Known You Seed, Taiwan)
23.	Southern Light	Introduction line (Known You Seed, Taiwan)
24.	Sugar Baby-Chunshin	Introduction line (Chung Shin Seed, Taiwan)
25.	Sugar Baby-Yates	Introduction line (Yates Seed Co., USA)
26.	Sunflower	Introduction line (Chung Shin Seed, Taiwan)
27.	Super Sweet 66	Introduction line (Spring Plough Agriculture, Hong Kong)
28.	SW 144	Introduction line (Chung Shin Seed, Taiwan)
29.	Ten-Bow	Introduction line (Chung Shin Seed, Taiwan)
30.	TM Dragon	Introduction line (Takii Seed, Japan)
31.	TM Lion	Introduction line (Takii Seed, Japan)
32.	Torino	F_1 hybrid WM 1800 × WM 1531 (PT Benih Citra Asia)
33.	Uranus	F_1 hybrid yellow diploid INR045 (F) from INRA, France × yellow diploid VB947 (M) from Technisem France (Technisem Asia Co. Ltd., Vietnam)
34.	Yellow Baby	Introduction line (Chung Shin Seed, Taiwan)
35.	Yun An Flower	F_1 hybrid WM 25.7.10.4.11.21.8 (F) × WM 7.6.12.23.5.9.20 (M) (Acegreen Seed Co. Ltd., Taiwan)

cultures in the sense that such fungal pathogen cultures do not have any bacterial and other fungi contaminations. The pure cultures were then used for *Fon* identification and inoculation studies.

Fon Identification

Fusarium wilt cultures were transferred into carnation leaf agar (CLA) for 3 weeks. Olympus Binocular™ Microscope was used to identify each of the isolated cultures. The identification of macroconidia and microconidia was done by referring to that of *The Fusarium Laboratory Manual* (Leslie and Summerell 2006). The identified cultures were then isolated and transferred into slanted media for extended use and time.

Fon Inoculation

Plant preparation for *Fon* inoculation

Seeds of each watermelon genotypes were planted for seedlings in trays containing sterilized media consisted of soil and dung manure (1:1). The young seedlings were carefully uprooted and transplanted to pots containing sterilized growth media to obtain a 14-day-old healthy watermelon seedling. Polybags of 15 cm × 15 cm were filled with sterilized media with the same composition one day before transplantation and *Fon* inoculation. The plant preparations were done in the Leuwikopo Greenhouse. Soil sterilization was carried out by autoclaving the soil media at 121°C for 30 min.

Fon isolate preparation for inoculation

Rice grains were rinsed with running tap water, air-dried, then sterilized at 121°C. Cultivation of *Fon* isolates was initially run inside the laminar air flow cabinet by transferring the *Fon* isolate culture into each of sterilized rice grain substrate and the rice grains containing the *Fon* isolates were then maintained at room temperature until used for inoculation.

Figure 1. Methods for obtaining symptomatic watermelon plant samples used for *Fusarium oxysporum* f. sp. *niveum* (*Fon*) isolation. (A, B) Sampling the Fusarium wilt symptomatic plants from field. (C) Cross section of infected crown showing brown circle in the xylem.

Inoculation of watermelon seedlings with *Fon* isolates

Fon inoculation was carried out at the Leuwikopo Greenhouse. Inoculum density of each *Fon* isolate was calculated weekly using a haemocytometer until cultures reached the targeted density of 10^5 cfu/g. Inoculation was applied by mixing inoculated substrate with sterile growing media (1:100) into 15 cm × 15 cm polybags containing sterilized growth media. The 14-day-old plant seedlings were then transferred from sterile seedling media into *Fon* infested growing media in polybags. A Completely Randomized Design was applied for each *Fon* isolate. The experiment was repeated two times with 15 plants from each combination. Plants were maintained in the greenhouse for incubation period (IP) and disease index (DI) observations.

IP and DI Observations

IP and DI were observed by using modified score from Chikh-Rouhu et al. (2008) with criteria: 0 = no symptom; 1 = beginning of wilting yet leaves were still green; 2 = almost total of leaves wilted; 3 = all leaves wilted with the green stem; 4 = plant died with necrosis on main stem and crown. IP was observed once in 2 days for 4 weeks to determine the number of days an isolate needs to cause symptomatic plants. DI was observed once a week for 1 month. DI was calculated by referring to Golenia in Swiader et al. (2002) formula as shown below:

$$DI = \frac{\sum (n \times v)}{N \times V} \times 100$$

with n = disease score (0–4), v = number of plants from n^{th} score, N = total observation per replication, and V = highest score (4).

DI was then used to define resistance category by following Martyn and McLaughlin (1983) method: 0–20% = strongly resistant; 21–50% = moderate resistant; 51–80% = slightly resistant; 81–100% = susceptible. Re-isolation of *Fon* was carried out to verify *Fon* infection by culturing the crown of scored symptomatic plants resulted from inoculation results of each *Fon* isolate tested in this study.

Data Analysis

IP data were recapitulated and averaged using Microsoft Office Excel 2003 for Windows. Meanwhile, DI data on the 4^{th} week after inoculation was computed separately for each isolate using SAS software to generate analysis of variance (ANOVA) and continued with Duncan's Multiple Range Test (DMRT) using SAS ver. 9.1.

RESULTS AND DISCUSSION

Characteristics of *Fon* Isolates

Cultures of three *Fon* isolates from symptomatic plant samples demonstrated different colony colors of FL and FP isolates compared to that of the previous isolated FK isolate. FK isolate formed a dark

purple colony, slightly differed from that of FP isolate that formed reddish dark purple colony, and FL isolate which showed dark, cream colony (Figure 2).

Species of the genus *Fusarium* were highly diverse since the genetic make-up and environmental variations created their morphological variations (Seo and Kim 2017). Microscopic identification based on *The Fusarium Laboratory Manual* (Leslie and Summerell 2006) and CLA media (Summerell et al. 2003) showed that FL isolate (Figure 3) and FP isolate (Figure 4) were different

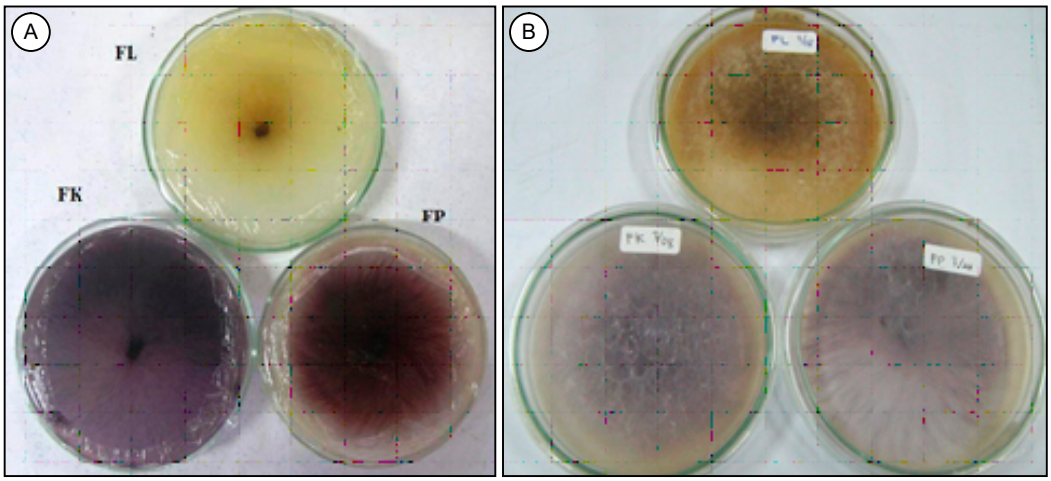

Figure 2. Growth characteristics of *Fusarium oxysporum* f. sp. *niveum* isolates derived from symptomatic watermelon plants collected from Karawang (FK), Lampung (FL), and Purwakarta (FP) on PDA media. (A) Underside part of the plated cultures. (B) Upper side parts of the plated cultures.

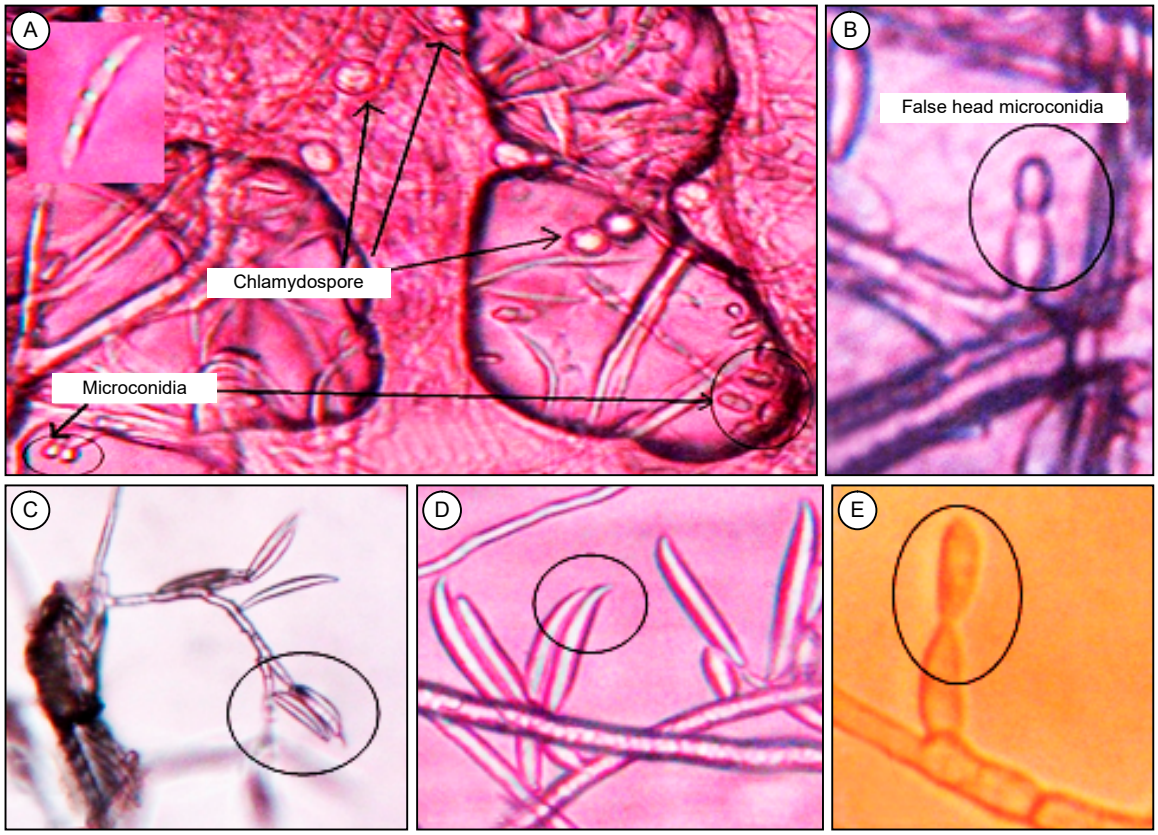

Figure 3. Identification of *Fusarium oxysporum* f. sp. *niveum* isolate FL derived from symptomatic watermelon plants collected from Lampung. (A) Chlamydospore and microconidia. (B, C) False head on microconidia. (D) Macroconidia with hooked apical cell on PDA media. (E) Macroconidia with monophialide on CLA media.

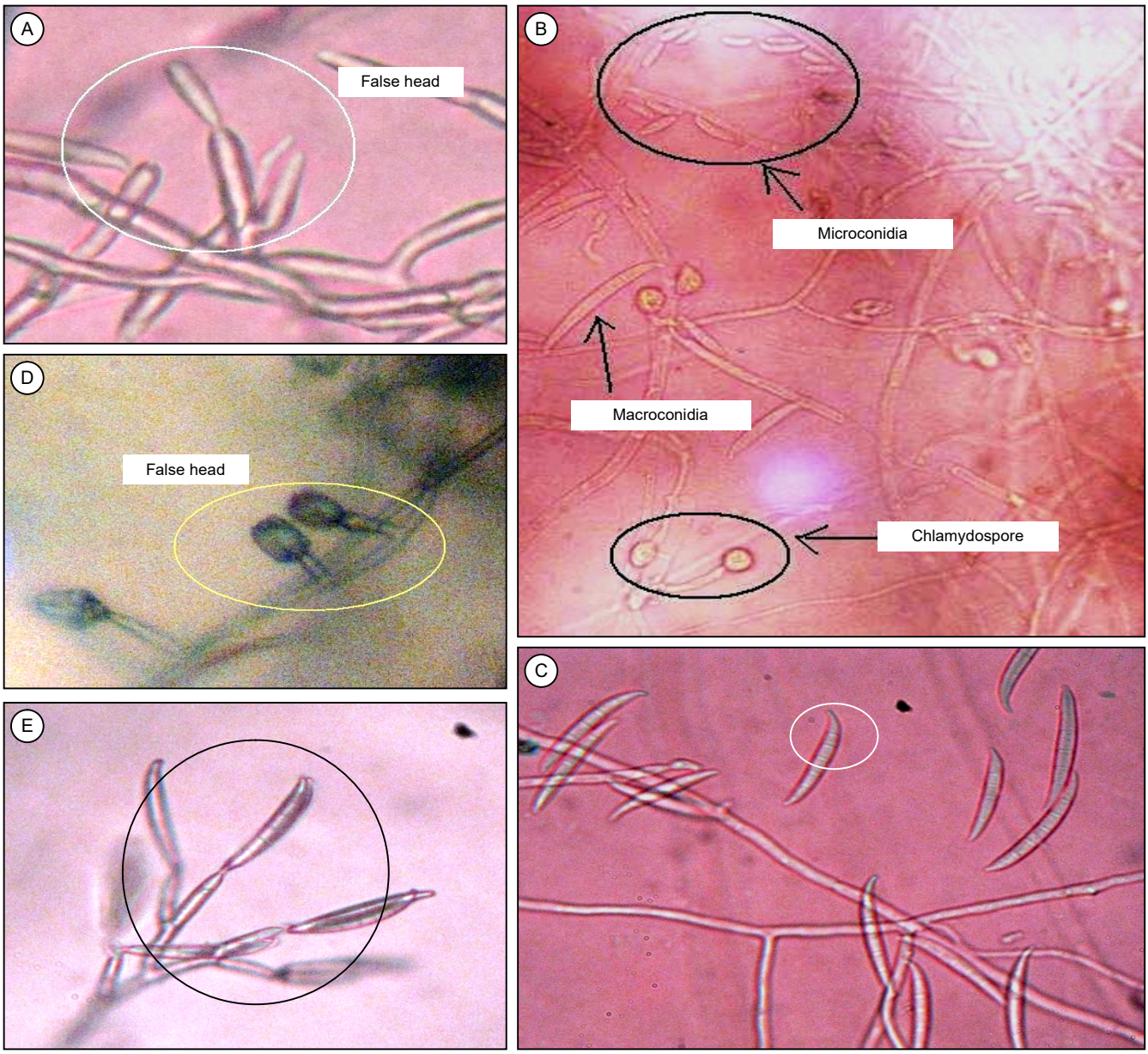

Figure 4. Identification of *Fusarium oxysporum* f. sp. *niveum* isolate FP derived from symptomatic watermelon plants collected from Purwakarta. (A) False head with monophialide. (B, C) Chlamydospore, microconidia, and macroconidia on PDA media. (D) False head aerial microconidia. (E) Macroconidia with branch and single monophialide on CLA media.

from *F. oxysporum*, based on observations of the structure of microconidia, false head on short monophialide, as well as chlamydospores.

Responses of Watermelon Genotypes to Three *Fon* Isolates

The *Fon* inoculation was carried out on 14-day-old plants (when the first leaves perfectly opened). Symptomatic plants as shown in Figure 5 had slightly wilted green leaves which turned to yellowish color followed by the weakened stem, stem (crown) necrosis, and finally collapsed. This phase of Fusarium wilt symptom was similar to the previous studies reported by Kurt et al. (2008) and Wechter et al. (2012). *Fon* can affect every stage of plant growth either in the seedling phase in the greenhouse or in older plants in the field. The infected seedlings showed stunted growth and wilted appearance followed by damping-off (Kleczewski and Egel 2011). *Fon* infection was initiated by the colonization of the root cortex to the xylem which blocks nutrition and water uptakes. At the end of the infection stages, *Fon* released lytic enzymes and toxin causing disease symptoms, such as necrotic, chlorosis, wilt, and plant

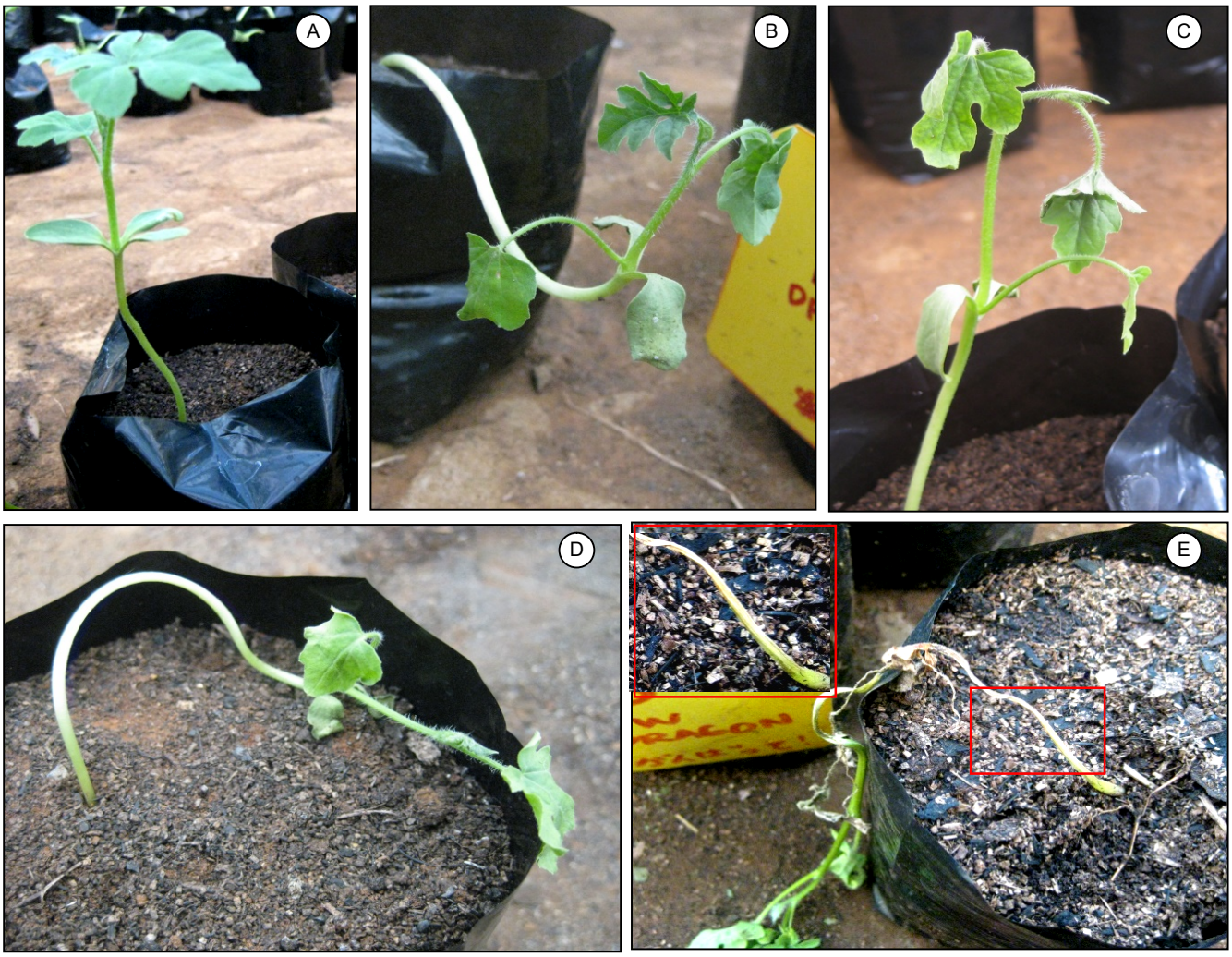

Figure 5. Inoculated watermelon plants showing *Fusarium oxysporum* f. sp. *niveum* infections. (A) No symptom appeared (score 0). (B) Plant was still vigorous with green and slightly wilted leaf (score 1). (C) The leaf wilted (score 2). (D) Plant started to collapse (score 3). (E) Plant collapsed with necrosis in stem (score 4).

death (Egel and Martyn 2007; Oumouloud et al. 2013).

The scoring system, which was adapted from *F. oxysporum* f. sp. *melonis* (Chikh-Rouhou et al. 2008) based on leaves and stem appearances, proved the ability to distinguish the changes of *Fon* infection symptoms of watermelon plants. Almost all of the observed plants expressed the changing stages of symptoms following the respective scores of 0, 1, 2, 3, and 4.

Incubation Period (IP)

IP data (Table 2) showed that no genotype seemed to escape the infection of any given *Fon* isolate despite of its resistance category. *Fon* is highly effective in colonizing and penetrating the crown of both resistant and susceptible watermelon plants (Zhou and Everts 2004). The IP also reflected the different reactions among genotypes against *Fon* isolates. Most of tested genotypes showed the longest IP of more than 3 weeks or 23 days after inoculation (DAI) when the genotypes were inoculated with FL isolate. This means that FL isolate required a longer period for causing symptoms on given tested watermelon genotypes. Meanwhile, FP isolate recorded the shortest IP (mostly 11–16 DAI) which indicated that this isolate elicited symptoms on plants faster than other tested isolates, FL and FK. This result also suggested different virulence ability among the three isolates to infect one genotype and *vice versa*. They differed in the genotypic reaction against one isolate, in terms of how long one genotype can endure for encountering the infection before expressing disease symptom.

Table 2. Average IP and PSP of watermelon genotypes infected with three *Fusarium oxysporum* f. sp. *niveum* isolates collected from Karawang (FK), Lampung (FL), and Purwakarta (FP).

No.	Genotype	FK		FL		FP	
		IP (DAI)	PSP (%)	IP (DAI)	PSP (%)	IP (DAI)	PSP (%)
1.	6372	>23	60.00	>23	26.67	11–16	35.00
2.	Amor	17–22	60.00	17–22	6.67	11–16	53.34
3.	Bangkok Flower	17–22	86.67	17–22	73.33	11–16	63.33
4.	Black Jumbo	>23	56.67	>23	63.33	11–16	55.00
5.	Charleston Gray	>23	50.00	17–22	40.00	11–16	70.00
6.	Diana Bangkok	>23	73.33	11–16	10.00	11–16	56.66
7.	Dragon Giant 145	17–22	63.33	>23	70.00	11–16	80.00
8.	Falcon	17–22	66.66	11–16	35.00	11–16	60.00
9.	Full Orchid	17–22	45.44	>23	10.00	17–22	50.00
10.	Golden Bright	17–22	73.33	17–22	76.67	17–22	43.33
11.	Grace	>23	43.33	>23	43.33	11–16	90.00
12.	Hitam Manis	11–16	49.99	>23	45.83	<10	50.00
13.	Jalur Madu	17–22	66.67	17–22	83.33	11–16	66.67
14.	King Dragon	17–22	66.67	>23	61.53	11–16	80.00
15.	Little Boy	11–16	80.00	17–22	33.33	11–16	66.67
16.	Mas Kuning	17–22	86.67	>23	83.33	11–16	13.33
17.	Milano	>23	40.00	>23	10.00	17–22	56.67
18.	New Dragon	>23	46.67	>23	6.67	11–16	50.00
19.	New Hope	>23	63.33	>23	36.67	11–16	80.00
20.	New Orchid	11–16	50.00	<10	57.14	11–16	60.00
21.	Nina	11–16	46.66	11–16	30.00	11–16	53.33
22.	Sky Mountain	17–22	76.66	17–22	100.00	17–22	93.34
23.	Southern Light	11–16	31.25	>23	46.67	>23	43.33
24.	Sugar Baby-Chunshin	11–16	73.34	>23	63.33	17–22	86.36
25.	Sugar Baby-Yates	>23	100.00	>23	93.00	11–16	43.33
26.	Sunflower	11–16	53.33	11–16	30.00	11–16	66.67
27.	Super Sweet 66	17–22	60.00	>23	50.00	17–22	36.67
28.	SW 144	17–22	83.33	17–22	20.00	11–16	80.00
29.	Ten Bow	11–16	50.00	>23	13.33	17–22	63.34
30.	TM Dragon	11–16	56.67	>23	39.28	11–16	63.33
31.	TM Lion	>23	38.46	>23	16.67	11–16	53.33
32.	Torino	17–22	60.00	>23	33.33	17–22	100.00
33.	Uranus	>23	36.67	17–22	6.67	11–16	40.00
34.	Yellow Baby	11–16	46.67	>23	18.18	11–16	53.33
35.	Yunan Flower	17–22	45.44	>23	16.67	17–22	50.00

IP = incubation period, PSP = percentage of symptomatic plants, DAI = days after inoculation.

When tested with FL isolate, of all 35 genotypes, New Dragon variety showed the longest IP (>23 DAI) with the least number of infected plants (6.67%), whereas New Orchid recorded the shortest IP with 57.14% of its population showing disease symptoms in less than 10 DAI. On the other hand, when tested against FP isolate, Southern Light recorded the longest IP (>23 DAI) with 43.33% of its population were infected, whereas Hitam Manis showed the shortest IP (<10 DAI) with 50% plants of this genotype were infected and showing disease symptoms. This different reaction was possibly caused by the genetic potential of each genotype to inhibit the pathogen colonization rates. Hudec and Muchová (2010) reported that there were different levels of pathogen aggressiveness among *F. oxysporum* isolates that were collected from different locations.

Disease Index (DI)

The calculation of DI showed variation of genotypic resistance responses against *Fon* isolates. The data was divided into four groups (Table 3) for easier interpretation. The first group (Group I) consisted of six genotypes that showed fairly good resistance against all tested *Fon* isolates, i.e. New Hope, Southern Light, Sky Mountain, Super Sweet 66, Uranus, and Yellow Baby. Group II consisted of seven genotypes that showed resistance only to both FK and FL isolates, i.e. Bangkok Flower, Charleston Gray, Full Orchid, Grace, Mas Kuning, New Dragon, and TM Lion. Group III consisted of 19 genotypes that showed resistance against only one of the *Fon* isolates. The last group (Group IV) consisted of three genotypes which appeared to be susceptible to all tested *Fon* isolates, i.e. New Orchid, Hitam Manis, and TM Dragon.

Table 3. Fusarium wilt DI (%) and resistance categories of 35 watermelon genotypes infested with three *Fusarium oxysporum* f. sp. *niveum* isolates collected from Karawang (FK), Lampung (FL), and Purwakarta (FP).

No.	Genotype	FK		FL		FP	
	Group I						
1.	New Hope	75.84$^{b–f}$	SR	40.84$^{d–h}$	MR	75.84$^{d–f}$	SR
2.	Sky Mountain	66.67$^{e–g}$	SR	25.84$^{e–h}$	MR	75.83$^{d–f}$	SR
3.	Southern Light	50.28^{g}	MR	31.67$^{e–h}$	MR	73.30$^{e–f}$	SR
4.	Super Sweet 66	72.50$^{c–f}$	SR	43.33$^{c–g}$	MR	67.50^{f}	SR
5.	Uranus	78.34$^{a–f}$	SR	10.00^{h}	R	76.67$^{c–f}$	SR
6.	Yellow Baby	78.33$^{a–f}$	SR	18.18$^{g–h}$	R	74.15$^{d–f}$	SR
	Group II						
7.	Bangkok Flower	79.17$^{a–f}$	SR	73.34$^{a–c}$	SR	81.25$^{b–f}$	S
8.	Charleston Gray	67.38$^{e–g}$	SR	30.00$^{e–h}$	MR	100.00^{a}	S
9.	Full Orchid	73.86$^{b–f}$	SR	23.34$^{f–h}$	MR	95.85$^{a–b}$	S
10.	Grace	73.34$^{b–f}$	SR	34.17$^{d–h}$	MR	93.30$^{a–c}$	S
11.	Mas Kuning	69.17$^{d–g}$	SR	10.00^{h}	R	100.00^{a}	S
12.	New Dragon	80.84$^{a–f}$	SR	16.67$^{g–h}$	R	96.65$^{a–b}$	S
13.	TM Lion	60.58$^{f–g}$	SR	16.65$^{g–h}$	R	100.00^{a}	S
	Group III						
14.	6372	83.33$^{a–e}$	S	31.67$^{e–h}$	MR	96.65$^{a–b}$	S
15.	Amor	94.17$^{a–c}$	S	20.00$^{g–h}$	R	100.00^{a}	S
16.	Black Jumbo	100.00^{a}	S	22.50$^{f–h}$	MR	93.30$^{a–b}$	S
17.	Diana Bangkok	100.00^{a}	S	13.32$^{g–h}$	R	93.30$^{a–b}$	S
18.	Dragon Giant 145	93.33$^{a–c}$	S	42.50$^{c–h}$	MR	85.85$^{a–e}$	S
19.	Falcon	84.15$^{a–e}$	S	12.50$^{g–h}$	R	96.65$^{a–b}$	S
20.	Golden Bright	81.67$^{a–f}$	S	37.50$^{d–h}$	MR	93.30$^{a–c}$	S
21.	Jalur Madu	100.00^{a}	S	27.08$^{e–h}$	MR	100.00^{a}	S
22.	King Dragon	95.00$^{a–b}$	S	40.38$^{d–h}$	MR	100.00^{a}	S
23.	Little Boy	94.64$^{a–b}$	S	24.17$^{e–h}$	MR	95.00$^{a–b}$	S
24.	Milano	93.34$^{a–c}$	S	13.32$^{g–h}$	R	100.00^{a}	S
25.	Nina	100.00^{a}	S	56.67$^{b–e}$	SR	92.50$^{a–c}$	S
26.	Sugar Baby Chunshin	90.00$^{a–d}$	S	30.00$^{e–h}$	MR	100.00^{a}	S
27.	Sugar Baby-Yates	85.42$^{a–e}$	S	65.00$^{a–d}$	SR	93.35$^{a–c}$	S
28.	Sunflower	90.84$^{a–d}$	S	53.34$^{b–f}$	SR	96.65$^{a–b}$	S
29.	SW 144	100.00^{a}	S	41.67$^{d–h}$	MR	96.65$^{a–b}$	S
30.	Ten Bow	86.67$^{a–e}$	S	19.17$^{g–h}$	R	81.73$^{b–f}$	S
31.	Torino	90.00$^{a–d}$	S	42.50$^{c–h}$	MR	86.67$^{a–e}$	S
32.	Yunan Flower	82.95$^{a–e}$	S	40.00$^{d–h}$	MR	90.00$^{a–d}$	S
	Group IV						
33.	New Orchid	100.00^{a}	S	92.86^{a}	S	93.30$^{a–c}$	S
34.	TM Dragon	85.84$^{a–e}$	S	90.18^{a}	S	100.00^{a}	S
35.	Hitam Manis	100.00^{a}	S	81.25$^{a–b}$	S	93.75$^{a–b}$	S

Numbers followed by the same letters in the same column are not significantly different according to DMRT α = 5%. Resistance category: R = resistant (0–20%), MR = moderate resistant (21–50%), SR = slightly resistant (51–80%), S = susceptible (81–100%).

Evaluation of genotypic reaction against different pathogenic isolates can be used to determine whether it is specific disease resistance or not (Liu and Anderson 2003). Thus, the genotype which proved to be resistant against isolate from one location can be susceptible if tested with other isolates from different areas (Latin 1993). Genotypes Sugar Baby and Charleston Gray showed the range of resistance category from moderate resistant, slightly resistant to susceptible when tested to each of *Fon* isolates. This result was similar to the previous study testing with the same genotypes (Tran-Nguyen and McMaster 2017). Unlike other *F. oxysporum* race which usually expressed resistance in discrete values, *Fon* frequently showed continued virulence (Zhou et al. 2010). Therefore, plants may expose a range of resistance categories, i.e. from highly resistant to susceptible (Leach et al. 2014). Our study also conformed to Patton (1955) who studied that the pathogen expressed a range of virulence level to resistance gene in the host plant. The external factors that potentially affect disease incident include the quantity of *Fon* inoculum infested in the soil (Kurt et al. 2008), sterility of growing media (Alves et al. 2008), inoculum substrate (Zhou et al. 2010), glucose and carbon contents of the inoculum (Srivastava et al. 2011), and temperature (Chen et al. 2013).

CONCLUSION

Different responses among 35 watermelon genotypes to three *Fon* isolates were demonstrated in this study as revealed by IP and DI. Most genotypes were slightly resistant to resistant against one *Fon* isolate but they were susceptible against other *Fon* isolates. Six watermelon genotypes that consistently showed resistance phenotypes against the three *Fon* isolates were New Hope, Sky Mountain, Southern Light, Super Sweet 66, Uranus, and Yellow Baby. These resistance genotypes are potentially used as parents in watermelon breeding programs for developing watermelon variety resistant to Fusarium wilt disease.

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AUTHOR CONTRIBUTIONS

NA is the main contributor who designed and performed the whole experiment and wrote the manuscript. SW and S are the member contributors who supervised, gave advices and guidance on the research design, and helped correction for the thesis from which this manuscript was written.

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