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## Characterization and Pathogenicity of *Fusarium oxysporum* as the Causal Agent of Fusarium Wilt in Chili (*Capsicum annuum* L.)

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3

Fusarium wilt is a serious disease attacking chili plants in Central Java which cause lost of chili productivity. Fusarium wilt is caused by pathogenic fungi *Fusarium oxysporum*, which is host specific. The objectives of this research were to characterize the pathogenic *F. oxysporum* as the causal agent of fusarium wilt in chili plants and to observe the virulence of the pathogen. Fungal pathogen was isolated from Tawangmangu as an endemic area of fusarium wilt in Central Java. The fungi was characterized morphologically and identified molecularly by its internal transcribed spacer regions (ITS regions). Pathogenicity test was done to observe the virulence of the pathogen. One pathogenic strain was isolated from Tawangmangu, Karanganyar and was identified morphologically and molecularly as *F. oxysporum*.

Key words: Characterization, pathogenicity, *Fusarium oxysporum*, *Capsicum annuum*

Layu fusarium merupakan salah satu penyakit tanaman yang sering dikeluhkan oleh para petani cabai di Jawa Tengah karena dapat menyebabkan gagal panen. Penyakit tersebut disebabkan oleh jamur *Fusarium oxysporum* yang bersifat spesifik inang. Penelitian ini bertujuan untuk menentukan karakter morfologis dan identifikasi secara molekular *F. oxysporum* patogen tanaman cabai dan mengetahui virulensi patogen tersebut. Patogen diisolasi dari Tawangmangu sebagai daerah endemis layu fusarium di Jawa Tengah. Jamur patogen dikarakterisasi secara morfologis dan diidentifikasi secara molekular menggunakan gen ITS. Isolat *F. oxysporum* yang didapat diuji patogenitasnya pada tanaman cabai. Hasil penelitian mendapatkan satu isolat jamur yang bersifat patogen serta secara morfologis dan molekular adalah jamur *F. oxysporum*.

Kata kunci : Karakterisasi, patogenitas, *Fusarium oxysporum*, *Capsicum annuum*

Chili is a potential horticulture in Indonesia and produced mainly in Java. Indonesia produced 954.360 ton red chili in year 2012 and 48% of the plantation was located in Java (Anonim 2013). Central Java has the second biggest of chili plantation but many plants have been attacked by fusarium wilt especially in the rainy seasons. The symptoms of fusarium wilt are wilting, vein clearing in younger leaflets, epinasty, stunting and yellowing of older leaves (Agrios 2005). According to Agrios (2005), fusarium wilt in plants was usually caused by *Fusarium oxysporum*. This species can infects more than 100 species of plants and causes the wilt symptoms. *Fusarium oxysporum* was the main disease in Thailand chili crops and one of the causal agents of wilting chili in India, China, and Indonesia (Ali 2006).

Pathogenicity of *F. oxysporum* in many plants have been studied for many decades. It is because the pathogen has a systemic reaction in plants that causes

the death of the infected plants. *Fusarium oxysporum* as the causal agent of wilting banana was reported by Groenewald (2005). Genetic diversity and pathogenicity of *F. oxysporum* was studied in Japanese onion (Dissanayake *et al.* 2009) and in Indonesian onion (Choiruddin 2010). Pathogenic *F. oxysporum* f. sp *vasinfectum* was determined in cotton roots by PCR based (Abd-Elsalam *et al.* 2006). The fusarium wilt had also been observed in melon (Herman and Perl-Treves 2007; Oumouloud *et al.* 2008). In Solanaceae, *F. oxysporum* was reported as the causal agent in tomato (Hibar *et al.* 2007; Jacobs *et al.* 2013), potato (Bayona *et al.* 2011; Du *et al.* 2012), and eggplant (Altinok *et al.* 2014). Studies of fusarium wilt in chili were done morphologically (Zahara and Harahap 2007), biologically (Nugraheni 2010), and enzymatically (Chaiyawat *et al.* 2008; Wongpia and Lomthaisong 2010).

Many chili crops in Indonesia have problems by fusarium wilt but the causal agent have not been determined yet. In this research a pathogen causing the

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fusarium wilt in chili was isolated and identified morphologically and molecularly. Molecular identification confirmed the morphological character. In this study, isolation of the pathogen was done from the endemic area of fusarium wilt in Tawangmangu, Karanganyar, Central Java.

## MATERIALS AND METHODS

**Plant Materials.** Wilting plant materials were collected from chili plantation in Tawangmangu Karanganyar. Treaty plants were TM999 cultivar (seeds produced by Seminis, Monsanto, Korea) and Gantari cultivar (seeds produced by Balai Penelitian dan Pengembangan Hortikultura Ngipiksari, Yogyakarta, Indonesia) grown in sterile soil.

**Fungal Isolation and Identification.** Fungi were isolated from the browning vascular of plants. The stem was cut on the border of brown vascular and healthy vascular. A five millimeter slice of the stems were surface sterilized in 70% ethanol for one minute, rinsed in sterile water and dried with tissue, and grown on the Potato Dextrose Agar (PDA). The cultures were incubated at 25 °C with 12 h dark and 12 h light cycle for 2 days. Each colony was transferred into new PDA and incubated for 5 days to get pure culture of isolate. Characterization was done based on the morphology of colonies and cells. When *Fusarium* species is present, the isolate was grown on Synthetic Nutrient-Poor Agar (SNA) to analyse the shape of macroconidia and microconidia. Identification was done by comparing the morphology with the atlas of *Fusarium* (Leslie and Summerell 2006; and Samson *et al.* 2008). A single spore of *Fusarium oxysporum* was used for further test (as master isolate).

**Pathogenicity Test.** The *F. oxysporum* was grown in Potato Dextrose Broth (PDB) for 4 days. Conidia densities were calculated with haemocytometer and adjusted to 10<sup>6</sup> conidia/mL. The inoculum was inoculated in chili plant by root dip method (Herman and Perl-Treves 2007; Karimi *et al.* 2010). One month old of healthy chili plants were taken from the soil. The root were rinsed in water, soaked in 1% Chlorox for 1 minute, rinsed with sterile water, and then soaked in fungal suspension for 30 minutes. This treatment were done for 10 plants. Roots of healthy plant were soaked in sterile water was done as control. Each plant was planted in sterile soil in polybags. Disease symptoms were observed every odd days after inoculation (DAI). Symptoms were remarked by the

stunting, chlorosis, and/or wilting of the leaves which determined by scoring. Score 0 = no symptom, 1 = lower height compared to control, 2 = lower height and chlorosis, 3 = 10% chlorosis and/or 10% wilting, 4 = 11 - 25% wilting, 5 = 26 - 50% wilting, 6 = 51 - 100% wilting and dead. The Disease Severity Index (DSI) was determined according to Wongpia & Lomthaisong, 2010:

$$DSI = \frac{\sum (\text{disease severity scale} \times \text{number of plants in each scale})}{\text{The highest numerical scale index} \times \text{total number of plants}}$$

The highest numerical scale index x total number of plants

Pathogen was re-isolated from the symptomatic plant. The pathogen was compared morphologically and molecularly with the master isolate.

**Molecular Identification.** Master isolate and isolate from symptomatic plant were identified molecularly. Molecular identification of fungi was done using ITS regions (Abd-El Salam *et al.* 2006; Toju *et al.* 2012). The ITS rDNA of the isolate was amplified by Polymerase Chain Reaction (PCR). Pair of primers used were ITS1 and ITS4. Sequences of the primers were forward ITS1: 5'-TCCGTAGGTGAACCTGCG G-3' and reverse ITS4: 5'- TCCTCCGCTTATTGATA TGC-3', with target size 540 - 570 bp. PCR was done with KAPA2G Fast PCR Kit. PCR program for ITS rDNA was 95 °C 3 minutes for pre-denaturation, 95 °C 10 second for denaturation, 52 °C 10 second for annealing, and 72 °C 10 second for polimeration. The cycle was repeated 39 times, and the final extension was 72°C 5 minutes. PCR products were verified in 2% agarose gel electrophoresis and the DNA was sequenced. The sequencing product was analysed by BlastN in the GeneBank database using MEGA 5.1 software.

## RESULTS

Tawangmangu was endemic of fusarium wilt in the rainy season 2013. There were wilting leaves and browning or discoloration in the stem of the diseased plants caused by fusarium wilt. One strain of *Fusarium oxysporum*, which was then called P1a, was successfully isolated from the diseased plants in Tawangmangu.

A specific fungi was successfully isolated from the stems. Figure 1 showed the morphological characters of the fungi. The observed morphological characters were as follows: colony with white cottony aerial

mycellium and purple on the reverse with 4 - 5 cm diameter at 5 days incubation on PDA. Conidia was grown from short phialid with a false head. Macroconidia was straight fusciform, pedicellate basal cell, 27 - 46 x 3 - 4,5  $\mu$ m, with 3 - 5 septates. Microconidia were abundant, ellipsoid or fusciform without or with 1 - 2 septates, 5 - 15 x 2,2 - 3,5  $\mu$ m. Chlamyospore was formed terminally or intercalary, single or in pairs. The morphology has previously been described by Leslie and Summerell (2006), and Samson *et al.* (2008), as the characteristics *F. Oxysporum*, thus confirming the

identification of the isolate.

The pathogenicity test showed that P1a caused wilting in chili. Figure 2 showed that the symptom increased significantly 15 DAI. At 19 DAI, the fungi caused wilting of TM999 cultivar and Gantari cultivar with DSI scores 0.4 and 0.63, respectively. So the P1a isolate was pathogenic to both cultivars. Re-isolation from the symptomatic chili plants obtained one isolate that is morphologically similar to P1a isolate. The isolate was named P1a'. Both isolates were identified molecularly.

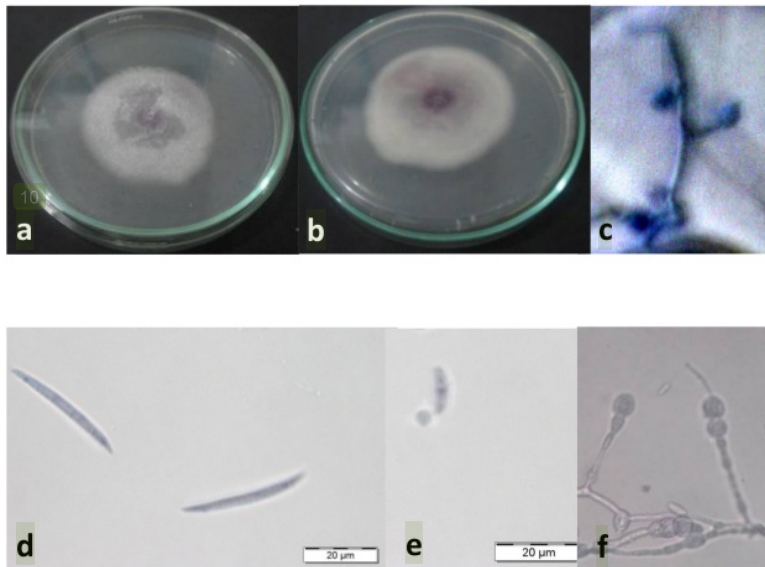


Fig 1 Morphology of *Fusarium oxysporum*. a. Colony, b. Reverse colony, c. Phialid , d. Macroconidia, e. Microconidia, f. Chlamyospore.

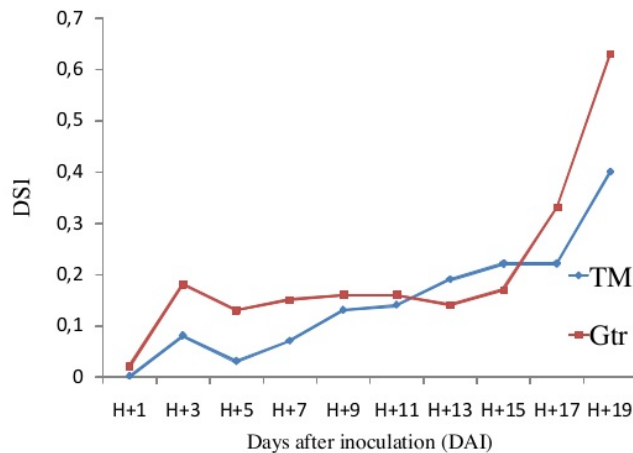


Fig 2 Disease severity index of the chili plants by *F.oxysporum*. TM: TM999 cultivar, Gtr: Gantari cultivar.

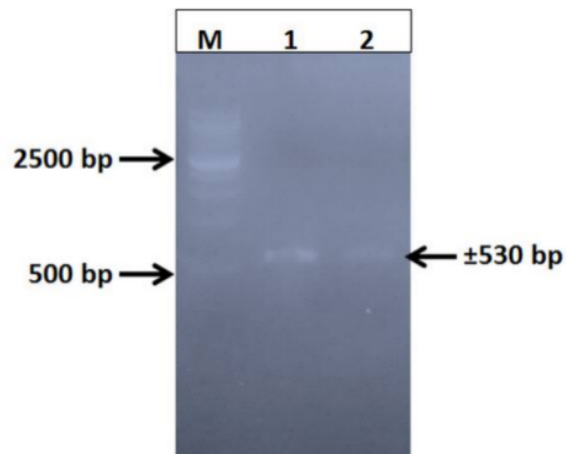


Fig 3 PCR products of *F.oxysporum*'s ITS rDNA. P1a (lane 1) and P1a' (lane 2) as 530 bp. M is DNA marker ladder (Geneaid).

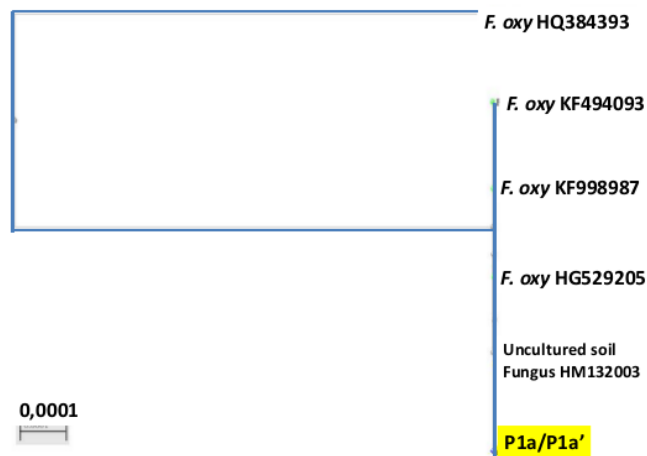


Fig 4 Phylogenetic tree of *F.oxysporum* pathogenic in chili (yellow box was P1a/P1a').

Molecular analysis showed similarity in the DNA sequences of P1a and P1a'. Figure 3 showed the PCR products of P1a and P1a' rDNA. The alignment of these sequences showed 100% similarity to *Fusarium oxysporum* accession number KF998987.1. Figure 4 showed the phylogenetic tree of the sequences with *F. oxysporum* rDNA sequences obtained from the GeneBank database.

## DISCUSSION

*Fusarium* wilt in Tawangmangu chili plantation covered 30–40% area and caused loss of crops. The

pathogen attacked plants in young and mature plants. Symptoms in young plants were stunting, chlorosis, wilting, and finally death. If the pathogen attacked a mature plant, flowers and fruits of the plant not develop normally. The disease was named fusarium wilt because wilting is the common observable symptoms. Wilting is actually a secondary symptom. The primary symptom was browning or discoloration in the basal stem, that is only observable by cutting the stem.

Macroscopical and microscopical characterization confirmed that the isolate was *F. oxysporum*. Molecular identification was needed to proof the name of the species. The rDNA ITS regions showed that P1a was the same as P1a', and confirmed the identity as *F.*

*oxysporum* KF998987.1. The rDNA ITS is a region commonly used in the fungal molecular characterization and taxonomic classification. However, it cannot be used in taxonomic classification below the species level, since will require more selective/specific sequences as well as specific primers for the sequence amplification, for example a pair of unique primer developed by Abd-Elsalam *et al.* (2006), which is specific to the sequence of *Fusarium oxysporum* f.sp *vasinfectum*.

Pathogenicity test showed that the *F. oxysporum* from diseased chili was a pathogenic fungi and caused the same symptoms in chili plants. The fungi cause stunting and wilting with or without yellowing. The wilting started from the older leaves and spread to the younger. The stem of the plants had vascular discoloration or browning in the transverse cutting. The wilting increased 15 DAI, when the pathogen had successfully infected the chili plants. The pathogen needed time to enter the plant root through wounding and therefore penetrated and colonized the vascular. Pathogen colonization in vascular bundle inhibited water and nutrient transport from soil to the shoot (Agrios 2005) so it caused wilting of the leaves. The time required for the *F. oxysporum* infection was similar to pathogenesis test in chili seedling (Suryanto *et al.* 2010) and wilted rocket plant (Srinivasan *et al.* 2011). The fungi needed 13 - 17 DAI to infect a plant and show symptoms. Due to its capability to grow on the nutrient medium, the *F. oxysporum* is classified as a non-obligate pathogen. The pathogen can grow and multiply on dead organic matter as saprophytic microorganism. In the case of *Fusarium*, they live in soil as chlamydo spores. The chlamydo spores were dormant in the soil until they met a specific host to grow. Planting the soil with soybean may not endanger the plants, but planting with chili will wake up the pathogen. So plants rotation is needed in the field to inactivate the pathogen.

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