Pelita Perkebunan 29(3) 2013, 210 - 219

Identification of a Disease on Cocoa Caused by *Fusarium* in Sulawesi

Identifikasi Penyakit oleh Fusarium pada Kakao di Sulawesi

Ade Rosmana[1*)], Hikmawati[1)], Muh. Zulfikar[1)], Asman[1)], and Dewi Fadillah[1)]

[1)]Cocoa Research Group, Faculty of Agriculture, Hasanuddin University, Makassar 90245 Indonesia
[*)]Alamat penulis (*corresponding author*): a2rosmana@yahoo.com
Naskah diterima (*received*) 22 Februari 2013, disetujui (*accepted*) 11 Maret 2013

Abstract

A disease presumed to be caused by Fusarium was observed in cocoa open fields with few or without shade trees. Within the population of cocoa trees in the field, some trees had died, some had yellowing leaves and dieback, and the others were apparently healthy. In order to demonstrate Fusarium species as the causal pathogen and to obtain information concerning the incidence of the disease, its distribution and its impact on sustainability of cocoa, isolation of the pathogen, inoculation of cocoa seedlings with isolates and a survey of disease has been conducted. Fusarium was isolated from roots and branches, and inoculated onto cocoa seedlings (one month old) via soil. Symptoms appeared within 3-4 weeks after infection. These symptoms consisted of yellowing of leaves beginning from the bottom until the leaves falldown, and browning internal of vascular tissue. Darkened vascular traces in the petiole characteristic of vascular-streak dieback infection were absent. The occurrence of Fusarium in the field was characterized by the absence of obvious signs of fungal infestation on root of infected trees, yellowing of leaves on twigs, dieback, and tree mortality in severe infestations. Disease incidence could reach 77% and in this situation it was difficult for trees recover from heavy infections or to be regenerated in the farm. The study proves that Fusarium is a pathogen causing dieback and the disease is called as Fusarium vascular dieback (FVD). Its development is apparently enhanced by dry conditions in the field.

Key words: Fusarium *sp.*, vascular disease, dieback, FVD, *Theobroma cacao* L.

Abstrak

Penyakit yang diduga disebabkan oleh Fusarium *diamati di pertanaman kakao terbuka dengan sedikit atau tanpa naungan. Dalam populasi tanaman di lapang, sejumlah tanaman mati, beberapa menunjukkan gejala kekuningan pada daun dan mati ranting serta yang lainnya tampak seperti sehat. Untuk membuktikan spesies* Fusarium *sebagai pathogen dan untuk memperoleh informasi mengenai kerusakan oleh penyakit serta distribusi dan dampaknya terhadap keberlanjutan kakao, maka isolasi patogen, inokulasi patogen, dan survei penyakit telah dilakukan. Fusarium diisolasi dari akar dan ranting serta diinokulasikan pada bibit kakao berumur satu bulan melalui tanah. Gejala muncul 3-4 minggu setelah inokulasi. Gejala ini berupa penguningan pada daun mulai dari bawah sampai daun jatuh dan pewarnaan coklat jaringan pembuluh. Pewarnaan hitam berkas pembuluh xilem yang merupakan khas infeksi* vascular streak dieback *tidak ditemukan. Keberadaan* Fusarium *di lapang dicirikan oleh tidak adanya tanda tanda kerusakan pada akar tanaman terinfeksi, penguningan daun pada ranting, mati ranting, dan kematian*

tanaman bila terjadi infestasi yang besar. Intensitas penyakit dapat mencapai 77% dan pada keadaan ini tanaman sulit disembuhkan dan kebun sulit untuk diremajakan. Studi ini membuktikan bahwa Fusarium *adalah pathogen yang menyebabkan mati ranting dan penyakitnya disebut sebagai* Fusarium-vascular dieback*. Perkembangan penyakit tampaknya distimulasi oleh keadaan kering di lapangan.*

Kata kunci: Fusarium *sp., penyakit vascular, mati ranting, FVD,* Theobroma cacao *L.*

INTRODUCTION

With more than 60% of national production, Sulawesi is an important cocoa producing area with great potential in supporting cocoa development in Indonesia (Ditjenbun, 2008). However, in the last ten years, productivity of cocoa farms in Sulawesi decreased significantly from around 1,200-1,500 kg dry bean/ha/year down to 400-700 kg dry bean/ha/year (Nasaruddin *et al*., 2007). This decrease was due to significant and extensive damage of cocoa plants as a consequence of mismanagement in production system and pests and diseases (Sjam *et al*., 2012).

The diseases commonly found are Phytophthora pod rot (PPR) and vascular streak dieback (VSD). VSD appeared in 2002 in Pinrang and Polman Regencies and from these regencies, the disease has spread rapidly to all part of Sulawesi (Rosmana, 2006; Guest & Keane, 2007; Sri-Sukamto *et al*., 2008). PPR has been known for a long time, but there is no data on when this disease first appeared in Sulawesi. The disease plays an important role in the reduction of cocoa production, not only in the wet season but also in the dry season when vectors such as ants exist in the field (McMahon & Purwantara 2004; Rosmana *et al*., 2010a; Rosmana *et al*., 2010b)

The disease reported here was presumed to be caused by Fusarium and was observed for the first time in Polman Regency in 2007 in open fields of cocoa with few or without shade trees. Among the population of cocoa trees in the field, some trees died, some had yellowing leaves and dieback, and the others were apparently healthy. This case has also occurred in mulberry, acacia, and silk tree (Rosmana & Wakman, 2004). Fusarium species are ubiquitous fungi found in the temperate and tropical areas of the world and cause a wide range of plant diseases that affect many crops, often with devastating socio-economic impact. Many species can colonize plants endophytically, an insidious process as it does not lead to symptom development, but contributes to a build-up in inoculum levels. However, stress may alter the relationship between a Fusarium endophyte and its plant host, leading to disease development (Burgess & Bryden, 2012). Cocoa trees, in open fields, could be stressed, notably in the dry season due to high intensity of sunshine. On other hand, Fusarium can be dominant on soil with low water content as occurs in the dry season (Rosmana *et al*., 2013). This research has been conducted to obtain information concerning the incidence of the disease and its impact on sustainability of cocoa and to prove Fusarium species as a causal pathogen of this disease.

MATERIALS AND METHODS

Isolation and Inoculation of Pathogen

The pathogen was isolated from cocoa roots and branches in the field predicted to

be infected by Fusarium. The roots of Polman origin and branches of Mamuju, Luwu, and North Luwu origin were cut into 1 cm sections and placed on sterile filter paper in petri dish, then incubated at room temperature. Surface sterilization was done by sequential immersion in 2% sodium hypochlorite, 70% ethanol and sterilized water before deposing on sterile filter paper. To obtain pure culture, fungi growing on sections of root and branches were transferred into PDA medium in petridish and then were identified.

One species of fungus predicted as a pathogen that occurred on roots and branches, respectively, was inoculated onto seedlings of about one-month old with a population of 10^6 spores/seedling through soil application. Each treatment included a control consisting of five seedlings, therefore the total was 15 seedlings. Five seedlings of three-months old were also planted in the field near the infected trees. The appearance of symptom and the growth of seedling were conducted in green house and similarly the seedlings in field were observed every day. After completing symptom observations, the pathogen was re-isolated from stem xylem to prove that the infecting pathogen was the same as the inoculating pathogen.

Survey of Disease

Survey of disease was done in Polman and Mamuju Regencies (West Sulawesi) and Luwu and North Luwu Regencies (South Sulawesi). Field samples in the first and the second regency were obtained from farms with a few or without shade trees, while field samples obtained in the third and fourth regency have gliricidia as shade trees. In Polman Regency, samples were taken from plantations with severe attack by the disease where the age of trees was more than 10 years. In Mamuju, samples were taken from plantation showing yellowing symptoms in their leaves, dieback, also showing VSD symptom, where the age of trees was about two years. While in Luwu and North Luwu, the samples were taken from plantations showing VSD symptom where the age of trees was around 18 months. The acreage of sampling was around 1 - 2 ha with a population of 1.000 - 2.000 trees. From these populations, ten percent of trees or 100 - 200 trees were sampled in five plots taken diagonally, each plot consist of 20 - 40 trees. The observation was done on dying trees, yellowing of leaves, and brown coloration of branch xylem. If symptoms were found even in branches, it was considered that one tree has been infected and intensity of disease was calculated using the formula of $I = A/B \times 100\%$ where I is intensity of disease, A is number of tree infected and B is the total of trees observed. For distinction from the symptoms caused by VSD disease on branches, the disease observed did not show any symptom of three black vascular traces on leaf scars and branches.

Observations were also made on the distribution mode of the disease, the effect on seedlings growing beside infected trees, and the effort the farmer made for management of infected trees.

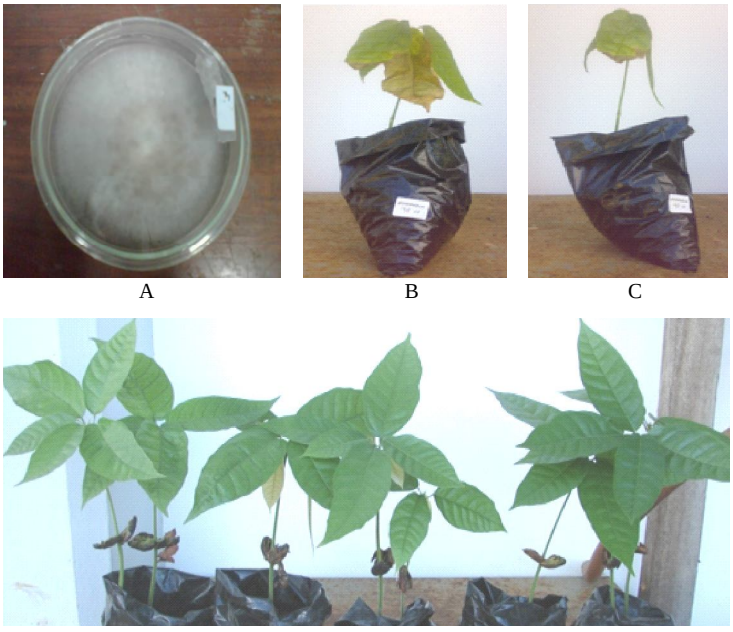
RESULTS AND DISCUSSION

Isolation and Inoculation of Pathogen

Isolation of fungi was done on roots from Polman and branches from Mamuju, Luwu and North Luwu. From roots species of *Fusarium*, *Trichoderma* and *Gliocladium* were isolated, while on branches, species of *Fusarium* (only in Mamuju), Basidiomycete fungus and two unidentified isolates

were obtained. Inoculation of *Fusarium* species both from roots and branches caused the same symptoms appearing 3-4 weeks after inoculation (Figure 1). This *Fusarium* can be re-isolated from infected seedling indicating that *Fusarium* is the causal agent of disease. The color of *Fusarium* colonies on PDA medium was creamy-white. The symptom showed by seedling externally was yellowing or drying of leaves starting from the bottom leaves, while internally it was browning of vascular tissue. The yellowing leaves finally fell down and due to this fall, leaves remained in the top part of seedling. The seedling exposed in the field gave the same symptom with retardation of their growth and no wilt symptom observed (Figure 2). So far, the disease caused by Fusarium have been given many names such as wilt, bayoud, pokkah boeng, stem rot, root rot, basal bulb rot, stem canker, dieback, Panama disease, malformation, fruit rot (Waller & Brayford, 1990). In the field on old cocoa, Fusarium cause also dieback similar to VSD disease, and not like the VSD pathogen, Fusarium can be found also on vascular tissue of old cocoa stem. Such symptoms both in green house and in field did not indicate any wilt, the disease is proposed to be called Fusarium vascular dieback. The same phenomenon was also observed on mulberry, acacia, albazia (Rosmana & Wakman, 2004) and durian (Rosmana, not published).

Species of Fusarium associated with dieback on cocoa as identified until now include *Fusarium solani, F. clamydosporum,*



A B C D

Figure 1. Fusarium species and cocoa seedling infected by Fusarium vascular dieback: *Fusarium* sp. grown in petri dish with white cream colony isolated from roots and branches (A); seedling infected with chlorotic or drying leaf beginning from lower leaves (B & C); seedling not infected with healthy green leaves (D).

Gambar 1. Spesies Fusarium *dan bibit kakao terinfeksi oleh* Fusarium-vascular dieback*; Fusarium sp. dengan warna putih krem tumbuh dalam cawan petri, diisolasi dari akar dan ranting (A); bibit terinfeksi dengan daun klorotik atau kering mulai dari bawah (B & C); bibit tidak terinfeksi dengan daun hijau sehat (D).*

Figure 2. Symptom of Fusarium vascular dieback on cocoa in field: the root of an infected tree is apparently healthy, with no indication of rot (above); seedling planted beside infected tree showing chlorotic leaves and retarded growing (below).

Gambar 2. Gejala Fusarium-vascular dieback *pada kakao di lapangan: akar tanaman terinfeksi tampak sehat, tidak ada indikasi busuk (atas); bibit yang ditanam di samping pohon terinfeksi memperlihatkan daun klorotik dan pertumbuhan terhambat (bawah).*

F. oxysporum, F. proliferatum, and *F. decemcellular* (Adu-Acheampong & Archer, 2011). Our isolate which has creamy-white color is correspond probably to one or more of above species, except *F. decemcellular*, as this species produce a mixture of pink and red pigments in the agar (Leslie & Summerell, 2006; Adu-Acheampong & Archer, 2011)

Distribution of Disease

The disease in the field was distributed randomly, not emanating from one point. Figure 3 represent the distribution on 100 trees population in Polman field showing the random mixture between dead trees, trees already infected, and apparently healthy trees. The data indicated that the pathogen was soil borne, already occurring in the soil and develops when the conditions around the tree are suitable for the growth and development of the pathogen. Fusarium is a genus of deuteromycetous fungi which are abundant in soils, in living plants, plant debris and other organic substrates as free-living saprophytes, pathogens, and endophytes (Summerell *et al*., 2003).

The existence of Fusarium-vascular dieback (FVD) disease in the field was characterized by no sign of fungal infestation on root of living trees (Figure 2), yellowing of leaf on twigs, dieback, and dying of tree in cases of severe infestation.

Other characteristics were that seedlings which were planted near infected trees were not well developed as noted above and also scions grafted on main stem did not succeed. Yellowing of leaf and dieback resembled the symptoms of VSD but without any presence of three traces both on leaf scars and on branches (Figure 4). When twigs were split infected xylem was visible as brown coloration within the vascular tissue.

In Polman Regency, among trees with more 10 years old, 77% had been infected by FVD; and some of them had already died. In Mamuju Regency, FVD had infected 22.3% trees of 2 years old, while in Luwu and North Luwu Regencies with trees of 18-month old, the occurrence of FVD disease was not detected by observation on branches (Table 1). Plant condition notably in old trees and environmental factors conditioned by shade trees affected development disease in the field. In Polman and Mamuju, fields had few or no shade trees and no soil cover, while in Luwu and North Luwu, fields had sufficient shade trees and were well managed.

Fusarium spp. is a soil-borne fungus that penetrates roots and can colonize plants endophytically, an insidious process that does not lead to symptom development, but contributes to a build-up in inoculum levels. Stress may alter the relationship between a *Fusarium* endophyte and its plant host, leading to disease development (Burgess & Bryden, 2012). Observation in the field indicated that the development of disease is very visible in dry season and its incidence is affected by the degree of shade provided by shade trees. When shade trees occur beside cocoa tree, the incidence is relatively low compared with trees in open fields without any shade trees. In this situation, two explanations are proposed: firstly, dry season and open field conditions will provide the necessary conditions for cocoa to receive high intensity of sun shine and this will lead to stress. Cocoa is a C3 crop that does not need a high intensity of sun shine. The old trees will be more affected by stress when exposed to high sunlight in the dry season. As noted above, stress conditions can enhance development of the disease caused by Fusarium species. Secondly, dry season and open field will reduce water content of soil significantly and this can lead to a biological vacuum in the soil; this means Fusarium species can be dominant while the other soil microorganisms do not persist under these conditions. Fusarium is capable of growing and developing in soil with limited water content (Toussoun, 1970). Study of *Fusarium* infection on mulberry at low (25 - 50%), medium (51 - 75%) and high (76 - 100%) water content indicated that highest incidence by *Fusarium* occurred at low soil water content and this incidence decreased with the increase of soil water content. At low water content, population of Fusarium was relatively high, while population of other soil microorganisms such as *Paecilomyces, Aspergillus, Trichoderma, Gliocladium*, and bacteria was relatively low. Contrary at a high water content population of *Fusarium* was one per ten of population at soil with low water content, while population of other microorganisms was eight time higher than population at low water content (Rosmana *et al*., 2013).

Besides FVD, often in Mamuju plantation, in the same tree, VSD was also observed. In this case, it is also possible that infestation of VSD in addition to dry season can cause stress to cocoa trees and enhance the development of FVD. In actual conditions, infected chlorotic leaves characteristic of VSD can develop progressively to non specific symptoms such as browning and necrosis (Guest & Keane,

2007; Purwantara *et al*., 2009). The complex of symptoms is hypothetically due to a different phase of the pathogen or intervention of other pathogens (Samuels *et al*., 2012; Parawansa, 2012). Therefore, species of Fusarium could be one of pathogens offering non specific symptom to VSD and it is supported by the fact that in area of high infestation of VSD, the most severe of disease incidence occurred in open field and in dry season. Laboratory study concerning this interference of Fusarium in contributing to complex symptoms of VSD is still underway. In areas with high infestation of disease such as Polman and Mamuju field, it is difficult to regenerate the cocoa plantation, because new seedlings planted in this field do not grow and develop as showed above and bud-wood grafted as side grafting also suffer before finally dying. In this situation, farmers normally abandon their field and this has been observed in many places in Sulawesi. Extreme dry season in Sulawesi frequently occurs and provides potential for infestation of disease caused by Fusarium. In other crops, the diseases have been reported on mulberry, acacia, silk tree (Rosmana & Wakman, 2004), durian (Rosmana, not published) and it is now shown to be found on cocoa.

In Luwu and North Luwu plantations, cocoa trees were shown just to be infested by VSD. These trees were still relatively young and have good vigor, and in addition, the field was well managed with gliricida shade trees. Condition permitting stress apparently enhance FVD infestation. From the time of seedling planting, the field should be well managed to avoid infestation of FVD disease. This includes the management of shade trees in order to protect cocoa trees from stress, notably in dry season and also the application of mulch and soil cover to avoid soil water content loss and to maintain the balance of soil microorganisms.

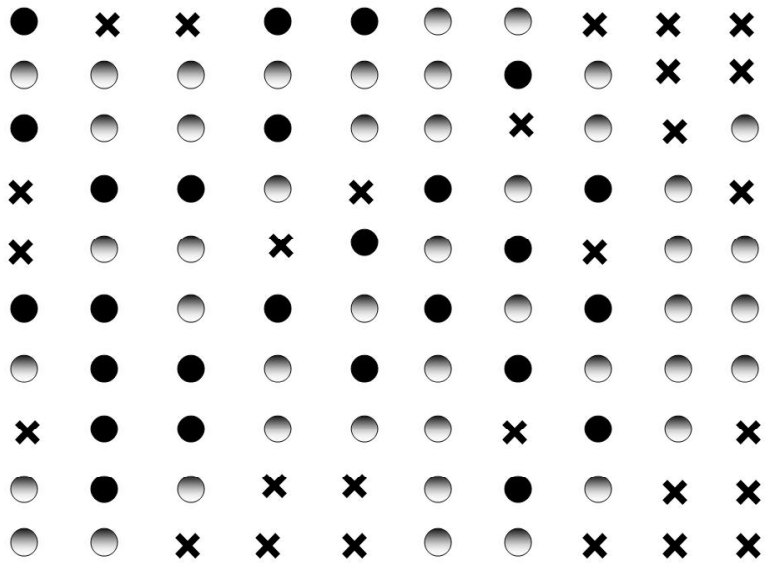


Figure 3. Distribution of cocoa tree infected by *Fusarium* vascular dieback on 100 trees in Polman field. Note: black circles = dead trees; grey circles = trees with dieback; cross = apparently healthy trees.

Gambar 3. Penyebaran tanaman kakao terinfeksi oleh Fusarium vascular dieback pada 100 pohon di Polman. Keterangan: lingkaran hitam = pohon mati; lingkaran abu-abu = pohon dengan mati ranting; silang = pohon yang tampak sehat.

Table 1. Incidence of *Fusarium* vascular dieback (FVD) disease on cocoa observed in Polman, Mamuju, Luwu, and North Luwu Regencies, Sulawesi

Tabel 1. Intensitas penyakit Fusarium vascular dieback (FVD) pada kakao yang diamati di Kabupaten Polman, Mamuju, Luwu, dan Luwu Utara, Sulawesi

Regencies *Kabupaten*	Age and clone of cocoa *Umur dan klon kakao*	Symptom *Gejala*	FVD disease intensity, % *Intensitas penyakit FVD, %*
Mamuju	2 years old tree; Sulawesi 01, Sulawesi 02, and local	Yellowing of leaves, dieback and VSD symptom	22.3
Polman	More than 10 years old tree;local	Yellowing of leaves dieback, dying of tree	77.0
Luwu	18 months old tree; Sulawesi 01 and M-01	VSD symptom	0.0
North Luwu	18 months old tree; Sulawesi 01 and M-01	VSD symptom	0.0

A

B

Figure 4. Branches infested by FVD without any presence of three traces both on leaf scars and on branches, but with brown coloration on vascular tissues (A) and yellowing leaf symptom infested by VSD with typically presence of three traces both on leaf scars and on branches (B)

Gambar 4. Ranting yang terinfestasi oleh FVD dengan tanpa adanya tiga noktah baik pada tangkai daun maupun pada ranting, tetapi terdapat pewarnaan coklat pada jaringan pembuluh (atas) dan gejala penguningan daun yang terinfestasi VSD dengan tiga noktah khas baik pada tangkai daun maupun ranting (bawah)

The information concerning the disease should be delivered to cocoa farmers as soon as possible, since so far they consider that FVD is VSD disease. False treatment would create fatal problems to the development of cocoa. Improvement of the growth environment such as planting of shade tree, mulching around main stem, covering soil with grass since seedlings are planted should be applied to inhibit the infection and development of FVD.

CONCLUSION

The disease showing symptoms of yellowing leaves and dieback resembling vascular streak dieback (VSD) disease and tree death in the field is caused by soil borne fungi, *Fusarium* sp. As this disease does not show any wilt symptom, it is called then as Fusarium vascular dieback (FVD) disease. In field, FVD can infect the same tree as VSD. With severe infestation of the disease, regeneration of the farm is difficult whether by side grafting of bud wood or by planting new seedlings.

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