

The Study and Early Evaluation of Resistance of Banana Accessions for Wilt Disease Caused by *Fusarium oxysporum* f.sp. *cubense* VCG 01213/16 (TR4)

Agus Sutanto^{a,b,*}, Sudarsono^a, Dewi Sukma^a, Catur Hermanto^b

^aDepartment of Agronomy and Horticulture, Bogor Agricultural Institute, Bogor, Indonesia

^bIndonesian Tropical Fruit Research Institute, Solok, Indonesia

*Corresponding author: Indonesian Tropical Fruit Research Institute,
Jl. Raya Solok-Aripan Km. 8, P.O. Box 5 Solok, West Sumatera Indonesia,

Tel: +62 755 20137, Fax: +62 755 20592

bagusutanto_02@yahoo.com

Abstract

Fusarium wilt is one of main diseases of banana in Indonesia. This disease has destroyed banana plantation in almost all parts of Indonesia and it is difficult to be managed by agronomic and chemical controls. However, some species/cultivars show tolerance or resistance to *Fusarium* wilt. It indicates that those species/cultivars have resistance genes in their genomic DNA. The evaluation of banana plants for *Fusarium* wilt resistance can be carried out artificially using young plants from tissue culture. The objectives of this research were to evaluate young acclimatized tissue culture plants for *Fusarium* wilt resistance and to study the resistance mechanism of plant to *Fusarium* wilt disease. The experiment used five banana accessions; there were Calcuta-4 (AAw), Ketan (AAB), Klutuk (BB), Kepok (ABB) and Ambon Hijau (AAA), and double compartment for planting system. Before planting on the plastic cup containing sterile sand medium, roots of the plantlets were dipped in 10⁶ conidia suspension of *Fusarium oxysporum* f.sp. *cubense* for five minutes. All of the cups containing inoculated plants were put on the plastic trays. The data were collected five weeks after planting. Base on the value of DSI (disease severity index) of RDI (rhizome discoloration index) and LSI (leaf symptom index), accessions will be categorized into highly susceptible, susceptible, tolerant and resistant. Result showed that Klutuk, Calcuta-4, Ketan and Kepok were categorized as tolerant, while Ambon Hijau was susceptible. All of tolerant accessions had symptom on leaves (LSI) and/or rhizome (RDI) at low level, and they still grew well.

Keywords: banana, resistance, fusarium wilt, early evaluation

Introduction

Banana is the most important fruit in Indonesia. Based on Ministry of Agriculture database (http://aplikasi.deptan.go.id/bdsp/hasil_kom.asp) banana contributed 31.10% of national fruit production in 2009, with the production reached 6.37 million ton. This potency can support three main programmes of agricultural development; food security, development of agribusiness and prosperity. However, the successfull of those programmes are constrained by pests and diseases development that affect banana plantation with vary disease intensity rank from 0.08-100% (Hermanto *et al.*, 2011). One of destructive banana diseases in Indonesia is fusarium wilt disease caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*). Fusarium wilt control using practical cultures such as chemical, soil treatments, crop rotation, organic amendments may reduce the severity of the disease but relatively difficult to be adopted commercially (Pegg *et al.*, 1993), therefore, the use of resistance cultivars is the best alternative for controlling this disease.

Field evaluation is the most reliable method for disease resistance selection, however, it requires high cost, manpower, space and facing the risk of environmental stress. Efforts to simplify the evaluation procedures have been carried out such as the use of young plant or *in vitro* calus as selected materials (Chand *et al.*, 2008), specific pathogen race or toxin as selective agent (Hadrami *et al.*, 2005), and screen house or *in vitro* condition as selection method (ŠVábová and Lebeda, 2005). The objectives of this

research were to evaluate young acclimatized tissue culture plants for fusarium wilt resistance and to study the resistance mechanism of plant to fusarium wilt disease

Materials and Methods

Planting Material, Foc Inoculation and Planting

The experiment used 10-15 cm in size of acclimatized *in vitro* plantlets of five banana accessions; Calcuta 4 (AAw), Ambon Hijau (AAA), Ketan (AAB), Kepok (ABB) and Klutuk Wulung (BB). Plantlets were gently uprooted and only those with healthy roots will be used for experiments by dipped in the *Foc* suspension (10^6 conidia/mL) for 5 min before replanted into cups containing sterile sand and placed in the trays for maintenance and observation. Plantlets were watered everyday and fertilized using nutrient solution (Hyponex®) every week.

Evaluation

Disease symptoms on leaves were recorded after the first two weeks, four weeks. Final evaluation was observed at fifth week based on the leaf symptom index (LSI) and rhizome discolouration index (RDI) (Mohamed *et al.*, 2001).

Scales for leaf symptom index (LSI) were:

1. No streaking or yellowing of leaves. Plant appeared healthy
2. Slight streaking and/or yellowing of lower leaves
3. Streaking and/or yellowing of most of the lower leaves.
4. Extensive streaking and/or yellowing on most or all of the leaves.
5. Dead plant.

Scales for rhizome discolouration index (RDI) were:

1. No discoloration of tissue of stellar region of rhizome or surrounding tissue.
2. No discoloration of stellar region of rhizome; discoloration at junction of root and rhizome.
3. Trace to 5% of stellar region discolored.
4. 6-20% of stellar region discolored.
5. 21-50% of stellar region discolored.
6. More than 50% of stellar region discolored.
7. Discoloration of the entire rhizome stele.
8. Dead plant.

After collecting data of LSI and RDI, the overall Disease Severity Index (DSI) for leaf symptoms and rhizome discoloration for each accession was calculated as follows:

$$DSI = \frac{\sum(\text{Number on scale} \times \text{Number of seedlings in that scale})}{\sum(\text{Number of treated seedlings})}$$

Furthermore DSI of LSI and RDI were translated into four categorize; resistant, tolerant, susceptible and highly susceptible (Table 1).

Table 1. Translation of DSI scales

DSI Scales for LSI	DSI Scales for RDI	Translation
1	1	Resistant
Between 1.1 and 2	Between 1.1 and 3	Tolerant
Between 2.1 and 3	Between 3.1 and 5	Susceptible
Between 3.1 and 4	Between 5.1 and 8	Highly susceptible

Results and Discussion

Early Evaluation of Banana Accessions toward *Fusarium* Wilt VCG 01213/16 (TR4)

Susceptible cultivar 'Ambon Hijau' produced disease symptom within two weeks after inoculation, with leaves chlorosis were started from older leaves to the younger leaves. Tolerant cultivar 'Klutuk Wulung' produced no symptom on leaves until five weeks after inoculation. The DSI of both LSI and RDI of five accessions and their susceptibility or tolerance status are shown in Table 2.

Table 2. The status of susceptibility or tolerance of five banana accessions

Samples	Calcuta-4		Ketan		Klutuk Wulung		Ambon Hijau		Kepok	
	LSI	RDI	LSI	RDI	LSI	RDI	LSI	RDI	LSI	RDI
1	1	1	1	1	1	1	2	4	2	2
2	1	1	1	1	1	1	2	2	1	1
3	1	1	1	1	1	1	1	1	1	1
4	1	1	3	3	1	4	4	5	1	1
5	1	1	2	3	1	1	2	4	1	1
6	1	1	2	2	1	1	2	5	1	1
7	3	3	2	3	1	1	3	5	1	1
8	1	1	1	1	1	1	2	4	1	1
9	2	3	2	3	1	3	2	5	1	1
10	1	1	1	1	1	1	2	4	1	1
11	1	1	1	1	1	1	2	3	1	1
12	1	1	1	1	1	1	2	3	2	2
DSI	1.25	1.33	1.50	1.75	1.00	1.42	2.17	3.75	1.17	1.17
Status	Tolerant		Tolerant		Tolerant		Susceptible		Tolerant	
Notes:	LSI	= Leaf Symptom Index								
	RDI	= Rhizome Discoloration Index								
	DSI	= Disease Severity Index								

Base on DSI of both LSI and RDI (Table 2), all accessions except Ambon Hijau were categorized as tolerant. Only one inoculated plant of Ambon Hijau had no symptom, while others showed symptom on leaves (LSI from 2 to 4) and rhizome (RDI from 2 to 5) (Figure 1B). Ambon Hijau is the member of Cavendish subgroup, which is naturally susceptible to *Foc* TR4. This finding was consistent with Hermanto *et al.* (2011), reported that 81% *Foc*-infected Ambon Hijau in Indonesia was caused by TR4 (VCG 01213/16). External symptom of infected leaves and internal symptom of Ambon Hijau were shown at Figure 1B. Meanwhile, only two out of twelve plants of Calcuta-4 showed *Foc* symptoms (DSI of LSI=1.25 and RDI=1.33, respectively) and the status of this accession was tolerant (Figure 1C). Calcuta-4 is wild-seeded species (*Musa acuminata* sp. *burmanica*) and they are often used for *Fusarium* wilt resistant breeding program (Tomekpe *et al.*, 2004).

Five out of twelve plants of Ketan showed symptom on leaf (LSI from 2 to 3) and rhizome (RDI from 2 to 3), however, DSI values of LSI and RDI were translated as tolerant (Figure 1E). Ketan also showed tolerant to *Foc* in some areas in Lampung, West Sumatera and West Java. Ketan had some synonyms in some places; Janten (Lampung), Jantan (West Sumatera), Uli (West Java), and Ketip (Nusa Tenggara Barat). This cultivar is popular as cooking banana. An interesting occurrence was shown by Klutuk Wulung. Two out of twelve plants produced symptom on rhizome (RDI=3), nevertheless, all plants showed no symptom on leaves (Figure 1A), therefore, DSI of LSI and RDI of this accession was translated as tolerant. Klutuk Wulung is seeded *Musa balbisiana* and scattered in Java, which usually people use leaves of it for wrapper and male bud flower for vegetable.

Another tolerant accession was Kepok, which two out of twelve plants showed symptom on leaves and rhizome (Figure 1D), and translation of DSI was tolerant. Kepok is very popular cooking banana and grows in whole part of Indonesia. Cases of infected plant of Kepok were found in West Java, Yogyakarta, Central Kalimantan and South Kalimantan. Kepok was not only infected by VCG 01213/16 (TR4), but also VCG 0120 and 01218 (Hermanto *et al.*, 2011).

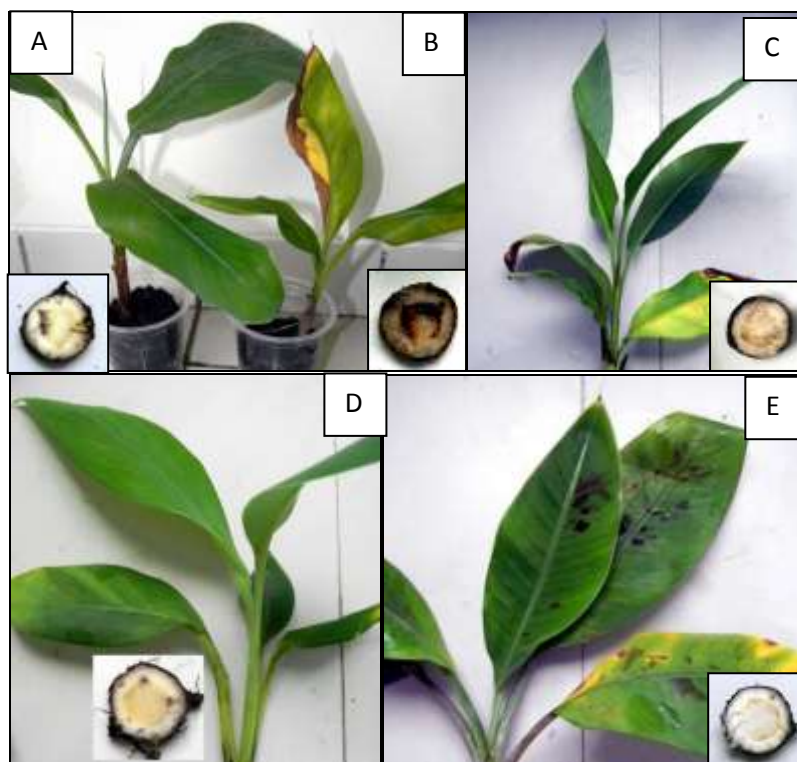


Figure 1. External (leaves) and internal (rhizome) symptoms of *Fusarium oxysporum* f.sp. *ubense* VCG 01213/16 on Klutuk Wulung (A), Ambon Hijau (B), Calcuta-4 (C), Kepok (D) and Ketan (E).

Resistance Mechanisms of Plant to *Fusarium* Wilt Disease

Resistance mechanism of plant against pathogen was started before the infection of pathogen into plant tissues. *Fusarium oxysporum* f.sp. *ubense* infects banana plant through root system. Since conidia attach hairy roots, they will germinate and penetrate into the epidermal cells of roots. Roots of resistant cultivar produce exudates that inhibit germination and growth of conidia; otherwise, exudates from roots of susceptible cultivar induce germination and growth of conidia (Li *et al.*, 2011).

Fungal pathogen is capable to penetrate plant roots through invading root epidermal cells directly, epidermal cell of root caps and elongation zone, natural wound in the lateral root base. During invasion, fungal hyphae produce cell wall degradation enzymes and penetrate to intercellular space, grow and develop branches and penetrate to other cells. Besides enzymes, fungal pathogen also produces micotoxins such as fusaric acid and beauvericin that affect trans-membrane electric potential, electrolyte leakage and respiration root cells (Pavlovkin 2006). Cell membrane damage causes the production of reactive oxygen species (ROS) and elicit the production of antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) (Kuzniak 2001), and transduction signal molecules that will trigger the production of pathogenesis related proteins such

as chitinase and β -1,3-glucanase. These enzymes will degrade fungal cell wall and inhibit growth and development of pathogen in the plant cells (Wu *et al.* 2008).

The case in Klutung Wulung, which infection symptom appeared in the rhizome but no symptom on the leaves (Figure 1A) indicated that resistance mechanism of plant against pathogen occurred. The development of fungi was localized only in part of rhizome and blocked for further expansion.

This evaluation technique was adequate for screening of *Fusarium* wilt resistant cultivars and the expression of the disease can be obtained within 6-8 weeks. Using small plants for evaluation can reduce space requirement when compared to field evaluation.

References

- Chand R, D Sen, KD Prasad, AK Singh, BM Bashyal, LC Prasad and K Joshi. 2008. Screening for disease resistance in barley cultivars against *Bipolar sorokiniana* using callus culture method. *Indian J Exp Biol*. 46:249-253.
- Hadrami AE, AE Idrissi-Tourane, ME Hassni, F Daayf, IE Hadrami. 2005. Toxin-based in-vitro selection and its potential application to date palm for resistance to the bayoud *Fusarium* wilt. *C. R. Biologies* 328:732–744.
- Hermanto C, A Sutanto, Jumjunidang, HS Edison, JW Daniells, WTO Neill, VGO Sinohin, AB Molina and P Taylor. 2011. Incidence and distribution of fusarium wilt disease of banana in Indonesia. *Proc Int ISHS-ProMusa Symp on Global Perspective on Asian Challenges. Acta Hort.* 897:313-322.
- Kuzniak E. 2001. Effects of fusaric acid on reactive oxygen species and antioxidants in tomato cell cultures. *J Phytopathol* 149(10):575-582.
- Li C, S Chen, C Zuo, Q Sun, Q Ye, G Yi and BZ Huang. 2011. The use of GFP-transformed isolates to study infection of banana with *Fusarium oxysporum* f. sp. *cubense* race 4. *Eur J Plant Pathol*. 131:327-340.
- Mohamed AA, C Mak, KW Liew, and YW Ho. 2001. Early evaluation of banana plant at nursery stage for *Fusarium* wilt tolerant. In: AB Molina, NH Masdek, KW Liew, Eds. *Banana Fusarium Wilt Management: Toward sustainable cultivation*, Los Banos, INIBAP-ASPNET. Pp:174-185.
- Pavlovkin J, I Mistríková, M Luxová, I Mistrík. 2006. Effects of beauvericin on root cell transmembrane electric potential, electrolyte leakage and respiration of maize roots with different susceptibility to *Fusarium*. *Plant Soil Environ*. 52(11):492–498.
- Pegg KG, Moore NY, Sorensen S. 1993. *Fusarium* wilt in the Asian Pacific region. In: Valmayor RV, Hwang SC, Ploetz RC, Lee SW, Roa NV, eds. *Proceeding of International Symposium Recent Developments in Banana Cultivation Technology*. Los Baños: International Network of Banana and Plantain/ASNET. Pp:225-269.
- Švábová L and A Lebeda. 2005. In Vitro Selection for Improved Plant Resistance to Toxin-Producing Pathogens. *J Phytopathol*. 153(1):52-64
- Tomekpe K, C Jenny, JV Escalant. 2004. A review of conventional improvement strategies for *Musa*. *Info Musa* 13(2): 2-6.
- Wu HS, XM Yin, DY Liu, N Ling, W Bao, RR Ying, YY Zhu, SW Guo, QR Shen. 2008. Effect of fungal fusaric acid on the root and leaf physiology of watermelon (*Citrullus lanatus*) seedlings. *Plant and Soil* 308: 255-266.

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