Wilt disease of banana (*Fusarium oxysporum* f. sp. *cubense*): Grouping of isolates in their physiological races

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Abstract. Fusarium wilt caused by *Fusarium oxysporum* f.sp. *cubense* (Foc) is a major disease on banana plants in the world and causes huge losses. The pathogen is very difficult to control, because the complexity of the pathogen that consisting in many races and can persist over 40 years without its host plant. This study was aimed to determine the physiological races and grouping of Foc isolates from several banana plantation areas in South Sulawesi. The study consisted of collection of *F. oxysporum* f.sp. *cubense* isolates and inoculation test onto plants with a concentration of 106 spores/ml. Barangan, Ambon and Kepok variety obtained from the tissue culture and Heliconia were used as test plants. *Fusarium oxysporum* f.sp. *cubense* suspension was applied by spraying on banana plant roots. Observation was conducted in the form of first symptoms appearance, leaf symptom development and observation of symptom in rhizome four weeks after inoculation. All of Foc isolates caused severe symptom on banana varieties tested and Heliconia. Based on this finding, it concluded that all isolates of Foc from Makassar, Bantaeng and Gowa district were classified into race IV.

1. Introduction

Banana (*Musa* sp.) is a popular fruit in the tropics and subtropics. Indonesia is one of the centre development diversities of bananas in tropical areas which has a high diversity of banana supplying for consumers. Banana market opportunities are still large enough for the domestic and foreign markets. Domestic bananas consumption reaches at 16 kg per capita per year and continues increasing by around 5.96% but it is not supported domestic supply. The import of fresh banana to domestic market is dominantly supplied from the middle East and East Asia [1, 2].

One of main factors causing insufficient banana supply in Indonesia especially South Sulawesi is banana wilt disease caused by soil-borne pathogen, *Fusarium oxysporum* f.sp. *cubense* [3, 4]. The pathogen has a wide host range, can spread to other healthy plants through the rhizome and water and survive in crops residues in the field [5]. Previously *F. oxysporum* f.sp. *cubense* form race 1 that infects tomato and banana [6-8]. Nowadays, *F. oxysporum* f.sp. *cubense* form race 4, is more resistant and infects all genomes of banana [8]. Resistant cultivar of the commercial banana such as Cavendish is used but the pathogen rises to a new variant of tropical race 4 (TR4). The presence of TR4 threatens global export and small-holder production of the Cavendish cultivar [9] allowing to limit the control of *F. oxysporum* f.sp. *cubense* particularly pathogen from non-infested areas. Perennial production of banana and the polycyclic nature of this disease hinders the development of other management strategies. Measures that are effective against annual or short-lived hosts of these diseases are usually
ineffective against Fusarium wilt of banana [10, 11]. The pathogen infects all stages of banana development. The pathogen is very difficult to control because of complexity of pathogen races living in soil [12]. The pathogenic fungi can persist in a period of 40 years without its host plant [13, 14]. Consequently, the need for eradication to limit the distribution of disease inoculum source is the last option [15], while attempt to design resistance plant is explored to avoid the disease particularly race TR4 [16]. According to Sunarjono [2] stated that tissue culture technique resulting banana plantlet with pathogen free. Unfortunately, the technology is very expensive, difficult to operate in banana plantation in wide areas. According to Saravanan et al., [17]; Svabova and Lebeda [18] reported that few species of bacteria such as *Pseudomonas fluorescens* as alternative biological agent control *F. oxysporum* f.sp. *cubense* by in vitro selection. Pan et. al., [3] stated a possible control of *F. oxysporum* f.sp. *cubense* by using endophytic bacterium *Burkholderia cepacia*.

This study aimed to determine the physiological races and grouping of *F. oxysporum* f.sp. *cubense* isolates from several banana plantation areas in South Sulawesi. The methods of treatment used is simple because *F. oxysporum* f.sp. *cubense* suspension was inoculated by spraying on banana plants roots. This is very useful information grouping *F. oxysporum* f.sp. *cubense* isolates based physiological races and management effort *F. oxysporum* f.sp. *cubense* in banana plantation at South Sulawesi, Indonesia.

2. Materials and methods

2.1. Research site
Research was conducted at Agriculture Biotechnology Laboratory, The Research Centre of Hasanuddin University, Makassar and Experimental Farm (Ex-Farm) Faculty of Agriculture Hasanuddin University. The important materials were used in this research: Potato Dextrose Agar (PDA), isolates of *F. oxysporum* f.sp. *cubense* from Makassar (M1,M2), Bantaeng district (BTG1, BTG2) and Gowa district (GW1, GW2), varieties of banana seedlings (Ambon, Barangan, Kepok), Heliconia, alcohol 70%, aquadest, organic fertilizers and soil.

2.2. Source of banana test plants
Test plants consisted of Barangan (AAB), Ambon (AAA) and Kepok (ABB) varieties obtained from the tissue culture propagation and planted in the Experimental Farm, Universitas Hasanuddin. Two months banana seedlings were chosen and planted in a polybag contain, soil, organic fertilizer and sand. The healthy Heliconia was obtained from ornamental plant trader.

2.3. Isolate collection from *F. oxysporum* f.sp. *cubense*
Isolate of *F. oxysporum* f.sp. *cubense* obtained from Agriculture Biotechnology Laboratory collection, The Centre of Research Universitas Hasanuddin, Makassar. Isolate of pathogenic fungi was inoculated inside PDA medium at petridish then incubation at temperature room about 7 days.

2.4. Inoculation of *F. oxysporum* f.sp. *cubense* at different banana varieties
The pathogenic fungi *F. oxysporum* f.sp. *cubense* suspension was inoculated by spraying on banana plants roots with 106 spores/ml added 100 ml distilled water before inoculation. The conidia density used 106 spores/ml based counting from haemocytometer under the binocular microscope. The concentration of *F. oxysporum* f.sp. *cubense* suspension counting with a formula:

\[
C = \frac{T}{N} \times 0.25 \times 10^6
\]

where:
C = Concentration;
T = The average number of conidia in five boxes;
N = The number of observation box.

The observation was conducted the form of appearance first symptoms, leaf symptom development and symptom observation in rhizome four weeks after inoculation. All of *F. oxysporum* f.sp. *cubense* isolates caused severe symptom on banana varieties tested and Heliconia.

The symptom observation of banana plants based Leaf Symptom Index (LSI) based category:
1) the healthy plant indicating no line or yellowing at leaves;
2) limited line or yellowing at below leaves;
3) presence the line or yellowing almost at below leaves;
4) yellowing almost at the leaves;
5) plant die

The rhizome observation was conducted at four weeks after inoculation based Rhizome Discolection Index (RDI) with category:
1) no change color at rhizome and another part surrounding it;
2) no change color at the edge of rhizome, the color change surrounding the roots;
3) ≥ 5% the edge of rhizome has change color;
4) 6 – 20% the edge of rhizome has change color;
5) 21 – 50% the edge of rhizome has change color;
6) > 50% the edge of rhizome has change color;
7) change color in all the part of rhizome;
8) plant die.

After observation LSI and RDI, the counting of Disease Severity Index (DSI) with formula:

\[
DSI = \frac{\sum(Number of scale \times Number of plant on a certain scale)}{\sum(Number plant in treatment)}
\]

The result of DSI translated into four category of plant type as shown in table 1. The isolate classification into the race based reaction of banana varieties [8, 19] is shown in table 2.

| DSI scale number for LSI | DSI scale number for LSI | Category |
|--------------------------|--------------------------|----------|
| 1                        | 1                        | Resistant |
| 2.1 - 3                  | 3.1 – 5                  | Susceptible |
| 3.1 - 4                  | 5.1 – 8                  | Very susceptible |

| Race Group | Category on Banana Varieties | Race Group | Category on Banana Varieties |
|------------|-------------------------------|------------|-------------------------------|
| I          | resistant - tolerant          | II         | resistant - tolerant          |
|            | Very susceptible              |            | susceptible                   |
|            | resistant - tolerant          | III        | resistant - tolerant          |
|            | susceptible                  |            | resistant – tolerant          |
|            | resistant - tolerant          | IV         | resistant - tolerant          |
|            | Very susceptible              |            | Very susceptible –           |

| Race Group | Category on Banana Varieties | Race Group | Category on Banana Varieties |
|------------|-------------------------------|------------|-------------------------------|
| Ambon (AAA)| resistant - tolerant          | Barangan (AAB)| resistant - tolerant |
|            | Very susceptible              |            | susceptible                   |
|            | resistant - tolerant          |            | resistant – tolerant          |
|            | susceptible                  |            | resistant – tolerant          |
|            | resistant - tolerant          |            | Very susceptible –           |
3. Results and discussion
In the beginning, disease symptom of \textit{F. oxysporum} f.sp. \textit{cubense} showed yellowing and wilt on the banana leaves. The symptom increasing every week and showed in the test plants i.e: Ambon (AAA), Barangan (AAB) and Kepok (ABB). The Musaceae ornamental plant Heliconia not showed yellow or wilt symptom on the leaves. The forming yellowing and wilt at test plants because \textit{F. oxysporum} f.sp. \textit{cubense} produce a dangerous toxin that avoid transport of water and nutrition inhibit spread around the plant. Based observation banana wilt caused by \textit{F. oxysporum} f.sp. \textit{cubense} showed the Leaf Symptom Index of banana and Heliconia leaves after inoculation (table 3).

Table 3. Number Leaf Symptom Index (LSI) of banana and Heliconia leaves after inoculation

| Isolate | Ambon | Barangan | Kepok | Heliconia |
|---------|-------|----------|-------|-----------|
|         | LSI   | RDI      | LSI   | RDI       | LSI   | RDI   | LSI  | RDI |
| M1      | 2.3   | 3.3      | 2     | 2.6       | 1.3   | 2.3   | 1    | 1.1 |
| M2      | 2.3   | 2.6      | 3     | 2         | 1.3   | 2.3   | 1    | 1.1 |
| BTG2    | 2.3   | 2.6      | 3.6   | 2.3       | 1.3   | 2.6   | 1    | 1.1 |
| BTG3    | 2.6   | 3        | 3.3   | 2.3       | 1.6   | 2.3   | 1    | 1.1 |
| GW1     | 2.3   | 3        | 2     | 2.3       | 1.6   | 2.3   | 1    | 1.1 |
| GW2     | 2.3   | 2.6      | 2.6   | 2         | 2.3   | 3     | 1    | 1.1 |

Tabel 3 show that at first week, number of LSI at Ambon variety reach 2.3 and increasing around 2.6 to 3 in the second week. In third week, the higher number of LSI at isolate BTG2 (3.6) and the lowest number showed at isolate GW2 (2.6). The number of LSI at Barangan variety relative stable at the first until second week, except isolate BTG2 reached 2 to 2.3. The increasing number of LSI at third week was observed only in M1 (2.6), BTG2 (3), BTG3 (3.3) and GW1 (2.2). Kepok variety was showed reaction relative resistant at first week. At the second week started increasing number of LSI especially interaction with isolate BTG2 reach 2.6. In third week, the higher number of LSI at isolate BTG2 (3), GW1 (3) and GW2 (3). Heliconia plant showed not significant result observation started at first until third week. The interaction with all of isolates showed there are not symptom at leaves because number of LSI is 1. Based observations, in first until third week the symptom of Fusarium wilt showed on the leaves and rhizome increasing after inoculation \textit{F. oxysporum} f.sp. \textit{cubense} at three banana varieties: Ambon (AAA), Barangan (AAB) and Kepok (ABB). According to Svabova and Lebeda [18] reported that plant pathogens produce substances that stimulate production of plant growth regulators and detected through developmental growth response of root and stem.

The findings at fourth week showed a variation on the number of Leaf Symptom Index (LSI) and Rhizome Discoloration Index (RDI) in number at banana leaves and Heliconia (table 4).

Table 4. Number of Leaf Symptom Index (LSI) and Rhizome Discoloration Index (RDI) at fourth week.

| Isolate | Ambon | Barangan | Kepok | Heliconia |
|---------|-------|----------|-------|-----------|
|         | LSI   | RDI      | LSI   | RDI       | LSI   | RDI   | LSI  | RDI |
| M1      | 3     | 3        | 3     | 5.6       | 3     | 3.6   | 1    | 1   |
| M2      | 2     | 2.6      | 3.3   | 3         | 3.6   | 3     | 1    | 1   |
| BTG2    | 3.6   | 3.6      | 3.6   | 5.3       | 4     | 5     | 1    | 1   |
| BTG3    | 4     | 3.3      | 3.6   | 5.5       | 3.3   | 2.6   | 1    | 1   |
| GW1     | 3     | 4.3      | 3     | 3.6       | 3.6   | 3.3   | 1    | 1   |
Table 4 described that Ambon variety (AAA) at fourth week, the number of LSI at isolate M1 (3), GW1 (3) and GW2 (3). The lowest at isolate M2 (2) and the highest at BTG3 (4). The highest number of RDI was showed isolate GW2 (5.6) and lowest number at M2 (2.6). Number of LSI at Barangan variety (AAB) isolate M1, GW1 and GW2 was showed 3 as the lowest number. The highest number was showed from isolate BTG2 (3.6) and BTG3 (3.6). It means that Barangan susceptible to isolate BTG3 and GW1. The number of RDI showed the highest isolate M1 (5.6) and the lowest number at M2 (3). The LSI at Kepok (ABB) at isolate M1 (3), M2 (3) and GW2 (3) was showed similar number. The highest scale showed isolated BTG2 (4) means that the plant susceptible to isolate F. oxysporum f.sp. cubense. The number of RDI, the higher scale was showed by BTG2 (5) and lowest RDI at BTG2 (2.6), respectively. Heliconia did not show any significant symptom based observation from first to fourth week. It was indicated no interaction between all of the isolate F. oxysporum f.sp. cubense because number of LSI and RDI at scale 1.

Table 4 shows that F. oxysporum f.sp. cubense caused wilt to banana leaves. In fact, number of LSI and RDI increasing after inoculation F. oxysporum f. sp. cubense. This is caused by impact of secondary metabolites that increasing plant growth. Genera Fusarium capable in synthetic process stimulate plant growth. Commonly Fusarium synthetic fusaric acid that toxic and disturb plant cell and protoplasm [20, 21]. In long term, fusaric acid inhibit plant metabolism and caused reduce water and nutrient in development process from root to all of plant part [22, 23, 20, 24]. According to Ploetz [16] stated that the banana plant was attacked by Fusarium wilt caused yellowing in leaves then the leaves drop from the tree. Plant contain F. oxysporum f.sp. cubense are unable bearing fruit or forming empty fruit. Disease of symptoms in plant cell forming brown or black lines appear on the longitudinal cross section of the stem. This symptom observed by naked eyes or magnifying glass.

Table 5 shows that banana varieties such as Ambon, Barangan and Kepok were susceptible to isolate M1 and M2. In contrast, the resistant plant showed at Heliconia. It meaning that M1 and M2 including at race IV. Similar result to another isolates such as BTG2, BTG3, GW1 and GW2 showed the same symptom attacked Ambon, Barangan and Kepok. The result showed that all isolates of F. oxysporum f.sp. cubense from Makassar, Bantaeng and Gowa regencies were classified into race IV.

Based result at Table 5, inoculation of F. oxysporum f.sp. cubense to all banana varieties including at race IV where banana varieties contain genome AAA, AAB and ABB susceptible to six isolates. The different result was showed at Heliconia, this plant seems resistant to F. oxysporum f.sp. cubense. According to Perez-Vicente et al., [4], race IV from F. oxysporum f.sp. cubense categorized as dangerous pathogenic fungi because of its capability to infect many banana varieties. Warman and Aitken [5] stated that F. oxysporum is a common fungal species with faster spreading and attacked many crops in worldwide. Nel et al., [25] reported that the specific characteristic of Fusarium as the ability of fungi living in rhizosphere then attacked root without visible symptom. The ability of Fusarium in colonization rhizosphere sometimes difficult to be detected in the beginning because Fusarium can survive many times in waste of harvest and soil.

Table 5. Determination of F. oxysporum f.sp. cubense races based on leaf and rhizome reaction, 4 weeks after inoculation.

| Isolate | Variety | Race |
|---------|---------|------|
|         | Ambon  | Barangan | Kepok | Heliconia |     |
|         | (AAA)  | (AAB)    | (ABB) |          |     |
| M1      | (+ )   | (+ )     | (+ )  | (- )     | IV  |
| Leaf    |         |          |       |          |     |
| Rhizome | (+ )   | (+ )     | (+ )  | (- )     | IV  |
| M2      | (+ )   | (+ )     | (+ )  | (- )     | IV  |
| Leaf    |         |          |       |          |     |
| Rhizome | (+ )   | (+ )     | (+ )  | (- )     | IV  |
| BTG2 Leaf | (+) | (+) | (+) | (-) | IV |
| BTG2 Rhizome | (+) | (+) | (+) | (-) | IV |
| BTG3 Leaf | (+) | (+) | (+) | (-) | IV |
| BTG3 Rhizome | (+) | (+) | (+) | (-) | IV |
| GW1 Leaf | (+) | (+) | (+) | (-) | IV |
| GW1 Rhizome | (+) | (+) | (+) | (-) | IV |
| GW2 Leaf | (+) | (+) | (+) | (-) | IV |
| GW2 Rhizome | (+) | (+) | (+) | (-) | IV |

Note: Very susceptible (++); Susceptible (+); Resistant (-).

4. Conclusion

Based on these findings, it is concluded that all isolates of *F. oxysporum* f.sp. *cubense* obtained from Makassar, Bantaeng district and Gowa district were classified into race IV.

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