The response of kenaf accessions to *Sclerotium rolfsii* the causal agent of damping-off disease

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**Abstract.** Damping-off disease caused by a fungus *Sclerotium rolfsii* is one of important diseases on kenaf, a fiber-producing crop. The disease could cause considerable yield losses on fiber production. The use of resistant varieties is the most effective control method for the disease. Understanding the characteristic of resistance of kenaf accessions is important to support the development of resistant varieties. Therefore, this research aimed to determine resistant characters of 15 kenaf accessions to damping-off disease caused by *S. rolfsii*. The research revealed that there was one accession (CI/77/17/37) showed moderately resistance to *S. rolfsii*. In addition, five accessions (CI/77/17/21, CI/77/17/25, CI/190/31, C2/210/50/1, and C3/222/2/57) were susceptible and the other accessions were very susceptible to *S. rolfsii*. It was expected that this information would be useful for kenaf breeders to develop resistant varieties of kenaf to damping-off disease.

Key words: Kenaf, *Hibiscus cannabinus*, *Sclerotium rolfsii*, resistance

1. **Introduction**

Kenaf is a fiber-producing plant that can be utilised for many areas of industries such as textile, automotive, pulp, etc [1]. Kenaf fiber is categorized as a long fiber which is suitable for production of high-quality paper, geo-textile, fiber board, bioplastics, and particle board. The use of kenaf fiber is expected to fulfill the need for raw materials for paper industries, as it has a shorter period of harvesting time rather than woody plant. Kenaf only needs about four months until harvested, in contrast, the woody plants can only be harvested after seven years [2]. In addition to utilize kenaf fiber for pulp and paper industries, currently, kenaf fiber is also developed for automotive industries as part of car body interior.

The production of kenaf fiber is restricted by infection of damping-off disease. This disease is caused by a fungus *Sclerotium rolfsii* and it becomes one of important diseases of kenaf. The infected plants would show brown to black rot of the lower stem which result in wilting of the whole plant. Moreover, there is also white fungal mycelial strands on the basal stem as well as on the soil surface around it and small, dark brown sclerotia. The disease can infect all stages of plant and it often occurs when plant initially infected by root-knot nematodes. Either seedlings or young plants infected by this fungus would be collapse. When the infected stalks were cut transversely or longitudinally it would be
brown on the xylem [3]. At moist condition, the fungus would turn to pinkish on the infected part of plants [4].

Currently, the control method for damping-off disease still relies on the use of chemical fungicides. Beside it would increase the production cost, it is also not environmentally friendly. The use of resistant varieties is believed to be the most effective control method for the disease. In addition, it does not have negative effects on the environment as well as another production cost [5]. Moreover, the use of resistant varieties is a crucial part of the integrated pest management in the future [6].

To develop resistant varieties of kenaf to S. rolfsii, it requires broad resistant accessions as source for hybridisation program. On the other hand, there is limited information of the resistant characters of kenaf accessions to S. rolfsii. Therefore, this research was conducted to determine the resistance of kenaf accessions to S. rolfsii infection.

## 2. Research Methods

The research was conducted at the phytopathology laboratories and screen house of Indonesian Sweetener and Fiber Crops Research Institute, Malang, Indonesia since May to November 2016. The materials used to include 17 kenaf accessions, S. rolfsii inoculum, polybags, NPK fertilizer, cow manure, and sterile soil.

Of 15 kenaf accessions and two check accessions (SM/021H for tolerant and CPI-072176 for susceptible accessions) were sowed on the sterile seedling beds (45 x 30 x 15 cm) containing sterile sand at the screen house. Each bed consisted of 40 seeds. Subsequently, the beds were layered with 50 g of S. rolfsii inoculum then covered with 0.5 kg of sterile sand. The research was arranged on Completely Randomly Design with three replicates.

The inoculum source of S. rolfsii was obtained by isolating infected kenaf plants on Potato Dextrose Agar (PDA) media. The fungus was subsequently purified and multiplied on PDA. Then, seven-day-old isolates were used for source of inoculum for inoculation.

Observation of damping-off disease was conducted by counting the infected plants every day until 37 days after inoculation (dai) to measure the percentage of incidence of damping-off disease. Then the level of resistance was categorized according to [15] as follow very resistant ( 1% plants infected), resistant (1.1-10.0% plants infected), moderate (10.1-20.0% plants infected), susceptible (20.1-50.0% plants infected), and very susceptible (>50% plants infected).

## 3. Result and Discussion

Incubation period of S. rolfsii infection on 17 kenaf accessions performed at Table 1. There was no significant difference of incubation period of S. rolfsii infection among 17 kenaf accessions, in which it ranged from 4.0 to 6.0 dai. The fastest period of incubation (4.0 dai) occurred on kenaf accession C2/210/50/1, in contrast, C3/222/2/54, C3/222/2/57 and C6/242/90 have similar incubation period (6.0 dai) which was the latest period. Incubation period means time needed by the pathogen to infect the host and result in the first symptom. Moreover, incubation period might indicate the level of resistance of kenaf accessions to S. rolfsii infection, in which, the longest incubation period might indicate that the accessions have high level of resistance to the infection. On the other hand, the fastest period suggests that the accessions are susceptible to the pathogen.

| Accession code   | Incubation period (dai) |
|------------------|-------------------------|
| CI/77/17/10      | 4.66                    |
| CI/77/17/21      | 4.66                    |
| CI/77/17/22      | 4.33                    |
| CI/77/17/25      | 5.00                    |
| CI/77/17/37      | 4.66                    |
| CI/190/31        | 4.33                    |
| C2/210/50/1      | 4.00                    |
The development of damping-off disease incidence on kenaf accessions is presented at Figure 1. It revealed that the incidence of damping-off has been seen since the first week after inoculation with the percentage of infection ranged from 10-80% (Figure 1). In this stage, the fungus *S. rolfsii* infected kenaf seedlings as a result the seedlings could not be able to germinate. It is generally known as pre-emergence damping-off. Most of accessions showed a significant increase of disease incidence since week 1 to week 7 except seven accessions including CI/77/17/21, CI/77/17/22, CI/77/17/37, CI/190/31, C2/210/50/1, C3/222/2/54 and SM/021H, in which the incidence of damping-off disease of those accessions were steady.

| Accession     | Disease Incidence (%) |
|---------------|-----------------------|
| C2/217/25     | 5,00                  |
| C2/224/13/31  | 5,00                  |
| C2/236/21/5   | 5,00                  |
| C2/236/21/15  | 5,33                  |
| C3/222/2/54   | 6,00                  |
| C3/222/2/57   | 6,00                  |
| C6/79/38/34   | 5,66                  |
| C6/242/90     | 6,00                  |
| SM/021 H      | 5,33                  |
| CPI-072176    | 5,00                  |

We also found that damping-off also occurred on asymptomatic plants. It could be identified by the discoloration of xylem when the stems were slashed (Figure 2a). On severe infection, the sclerotia was found at the infected plants (Figure 2b).

**Figure 1.** Development of damping-off incidence on 17 kenaf accessions
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Figure 2. Discoloration of xylem on asymptomatic plants on the left side and healthy stem on the right side (a) and development of sclerotia on severe infection (b)

The damping-off incidence on 17 kenaf accessions varied from 20.00 to 90.17% (Table 2). Kenaf accession CI/77/17/37 has the lowest incidence (20%), in contrast C6/79/38/34 was the highest incidence (90.17%). In addition, the resistant level of kenaf accessions to S. rolfsii infection ranged from moderately resistant to very susceptible. Kenaf accession CI/77/17/37 was categorized as moderately resistant, CI/77/17/21, CI/77/17/25, CI/190/31, C2/210/50/1 and C3/222/2/57 were grouped into susceptible, and the rest accessions were very susceptible to S. rolfsii infection.

| Accession | Disease incidence (%) | Level of resistance |
|-----------|------------------------|---------------------|
| CI/77/17/10 | 75.83                  | Very susceptible    |
| CI/77/17/21 | 48.19                  | Susceptible         |
| CI/77/17/22 | 70.83                  | Very susceptible    |
| CI/77/17/25 | 35.83                  | Susceptible         |
| CI/77/17/37 | 20.00                  | Moderate            |
| CI/190/31  | 39.62                  | Susceptible         |
| C2/210/50/1 | 33.75                  | Susceptible         |
| C2/217/25  | 77.50                  | Very susceptible    |
| C2/224/13/31 | 76.67                 | Very susceptible    |
| C2/236/21/5 | 86.87                  | Very susceptible    |
| C2/236/21/15 | 58.33                 | Very susceptible    |
| C3/222/2/54 | 58.66                  | Very susceptible    |
| C3/222/2/57 | 37.50                  | Susceptible         |
| C6/79/38/34 | 90.17                  | Very susceptible    |
| C6/242/90  | 67.50                  | Very susceptible    |
| SM/021 H (Tolerant check) | 41.47             | Susceptible         |
| CPI-072176 (Susceptible check) | 75.00           | Very susceptible    |

The level of resistance was classified according to Mandal (1988) category.

Discoloration of xylem on infected plants indicated with the change of xylem into brown was caused by the release of polyphenol secreted by the pathogen into plant tissues. It was subsequently polymerised into brown-melanine with the assistance of polyphenol oxydase produced by the plant host. At a severe infection, the lower stem would be rot and the whole plant wilt and collapsed [7].

Accessions with low incidence probably have defense response mechanism that can protect them from pathogen infection such as cytoplasmic defense reaction that can alter cytoplasm into small and
solid granules then break the fungal mycelia, as a consequence, the pathogen invasion ceased. Another possible defense mechanism was the change of plant cell wall structural morphology such as lignin formation as a result of the response of plant tissues to pathogen infection [8]. Defence mechanisms can also occur because of limited nutrition available for pathogen growth and development. In addition, the plant tissues can also produce chemical compounds that can inactivate toxins or enzymes secreted by the pathogen. The apple plant inoculated with a fungal pathogen Fusarium oxysporum would increase total content of phenol that can reduce pathogen invasion. Moreover phenol content and activity of polyphenoloxidase (PPO) climbed significantly when rosella infected with the pathogen. These activities are responsible for the resistance mechanisms of plant to pathogen infection [9]. According to [10] phenol content within plant tissues related to defense mechanism to pathogen infection. In addition to phenol content, lignin formation could also reduce the infection, in which kenaf has high-content of lignin on its stalks[11]. Other phenolic compounds such as stilbenes, coumarin, (neo) lignan, phenylpropanoid conjugate, and flavonoids [12] were identified as group of phytoalexins which have antimicrobial activities and involved in plant defense responses [13].

Another defense mechanism of plant to pathogen invasion is formation tylose within plant tissues. [14] Revealed that tylose is over growth of protoplasts of parenchyma cells that bulge through the circular bordered pits of vessel members and block water movement. Tylose consists of cellulose wall with varied size and amount which are able to impede translocation of water and cell nutrition. Hence prevent the pathogen to penetrate plant tissues.

4. Conclusion
There was one moderate accession (CI/77/17/37) of kenaf to S. rolfsii infection, five susceptible accessions (CI/77/17/21, CI/77/17/25, CI/190/31, C2/210/50/1 and C3/222/2/57), and the other nine accessions were very susceptible to S. rolfsii infection.

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