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SHORT COMMUNICATION

Tar spot disease of sorghum plants caused by *Phyllachora* sp. in Bogor, Gunung Kidul and West Lombok, Indonesia

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ABSTRACT

The sorghum (*Sorghum bicolor*) plant is highly tolerant and adapted to drought. It has the potential to be developed and planted on marginal land in Indonesia. Tar spot disease is one of the obstacles to sorghum cultivation. In Indonesia, there is no report yet about this disease, especially on sorghum. To achieve optimal disease management, information on disease incidence and severity levels, as well as pathogen tar spot disease identification, are required. The aims of this study were to morphologically identify the pathogen and determine the level of incidence and severity of tar spot in sorghum cultivation areas in Bogor Regency, Gunung Kidul Regency, and West Lombok Regency. The disease observations were carried out on 17 sorghum varieties from the three areas with no experimental design. The results of the observation of the tar spot disease incidence in 17 varieties of sorghum from Bogor, Gunung Kidul, and West Lombok were 100%. The level of tar spot disease severity varied from 32.4% in the Latu Keta sorghum variety to the highest of 87.9% in the Samurai sorghum variety. The macroscopic and microscopic observation results of tar spot on sorghum plants showed that the cause of the tar spot disease was the *Phyllachora* fungi. This is the first report of a tar spot on sorghum caused by *Phyllachora* sp. in Indonesia.

Key words: first report, morphology identification, severity

INTRODUCTION

Sorghum (*Sorghum bicolor*) is the cereal crop that is currently the fifth most agriculturally important crop in the world in terms of total production and planted area (Gladman et al., 2019). Biruma et al. (2012) reported that sorghum is highly tolerant to drought. According to Kong et al. (2018), these tolerances allow sorghum to be grown on marginal lands under global warming environmental conditions. In Indonesia, sorghum is one of the crops developed to meet human and animal food needs as well as industry (Soenartiningsih et al., 2013). Sorghum also has wide adaptability, so it can be grown on dry land and has the potential to be developed and cultivated in Indonesia (Santosa & Human, 2009).

Sorghum cultivation is constrained by pest and disease attacks (Soenartiningsih et al., 2013). Breeding can be used to improve sorghum plants against pests and diseases. Selection of sorghum accession which has resistance to pathogen infection against the disease is needed as the genetic source for breeding. For those purposes, comprehensive information about the disease is needed. Symptoms of attack, morphological characteristics, modes of transmission, levels of disease incidence and severity, and disease epidemiology must be known. This information is also needed in order to determine the disease management (Soenartiningsih et al., 2013) with the appropriate technique or method in the breeding materials selection. On the other hand, if we know the numbers of disease incidence and severity, we can assume that the pathogen can have a significant effect on the plant, especially on plant health and plant production. After we know the disease incidence and severity, we shall proceed to the identification of that pathogen.

Identification of pathogenic fungi that cause plant diseases is needed to obtain optimal disease control management (Rustiani et al., 2015). This identification is especially for the pathogen fungi that have never been known before, such as *Phyllachora* causing tar spot disease in sorghum. There was no report about tar

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spot disease in sorghum plants, especially in Indonesia. Appropriate disease control management must be supported by an adequate diagnosis of the pathogen. Identification of the pathogens according to IPPC (2009) is a diagnostic action to determine the profile of the pathogen that can be used for plant protection purposes. Related research about the pathogen identification through morphological characterization of the *Phyllachora* has been widely reported on other plants of the Poaceae family, but there have been no reports on sorghum plants, especially in Indonesia. The information above is not sufficient in Indonesia, so it is necessary to conduct research related to the identification of morphology and molecular *Phyllachora* in sorghum plants in Indonesia.

The initial identification of the *Phyllachora* sp. causing tar spot disease and pathogenic fungi generally can be done by morphological identification of the fungi (Tonge et al., 2014). Morphological identification was carried out by both macroscopic and microscopic observations. Macroscopic observations can be made by observing the visible symptoms, the shape, size of the spots, and other symptoms around the spots. Microscopic observations included observations of the mycelium, the wall of perithecium with its shape and size, and the shape and size of the ascus and ascospores (Pereira et al., 2010; McCoy et al., 2018). This research reported the incidence and severity of tar spot disease caused by *Phyllachora* sp. on sorghum plants in sorghum cultivation areas in Bogor Regency, Gunung Kidul Regency, and West Lombok Regency. This report is also the novelty of the research because there have been no reports before of this tar spot disease on sorghum in Indonesia. This research also reported the results of the morphological identification of the *Phyllachora* sp. on sorghum, thus confirming that this tar spot disease is caused by the *Phyllachora* sp.

MATERIALS AND METHODS

Research Site. This research was conducted in the Genomic Laboratorium, Research Center of Genetic Engineering, National Research and Innovation Agency, Cibinong, Bogor, West Java. Observations and sample collection were taken in sorghum cultivation area in Cibinong, Bogor Regency, West Java Province (6°29’38”S, 106°50’48”E); Playen, Gunung Kidul Regency, Special Region of Yogyakarta Province (7°57’50”S, 110°30’48”E) and Narmada, West Lombok Regency, West Nusa Tenggara Province (8°35’52”S, 116°13’8”E).

Sample Collection. Sorghum leaves with tar spots were collected directly from the field without any experimental design. Fresh leaves were cut and placed between banana pseudostems to preserve the freshness of leaf samples until the laboratorium.

Observation on Disease Incidence and Disease Severity. Disease incidence observations were carried out on several sorghum plants which represented the three areas of sorghum cultivation. The disease incidence and severity of the Konawe Selatan, Super 1 and Pahat varieties from the sorghum cultivation area in Bogor, then on the Bioguma and Samurai varieties in Gunung Kidul and on the Latu Monca, Latu Keta Na’e, Latu Keta, Latu Kaca, Gando Bura and Gando Keta varieties in West Lombok. For the disease incidence, the number of plants with disease and the total number of plants were observed. The percentage number of disease incidence can be calculated using the Zadoks & Schein formula (1979).

$$DI = \frac{n}{N} \times 100\%$$

DI = Disease Incidence (%);
n = Number of diseased plants;
N = Number of plants that observed in each cultivation line.

Disease severity was calculated by selecting several plants randomly from each variety line, marking each sample plant, then observing the sample plants that were attacked by the disease and scoring them according to the predetermined disease scoring. The tar spot disease score was referred to by Loladze et al. (2019) with disease rating scores from 1 to 5. Score 1 denotes the highly resistant or close to immune reaction with nearly 0% of infected leaves and no visible *Phyllachora* stromata; and score 2 denotes the plant that was resistant to moderately resistant reaction with 1% to 25% of leaf area affected by some scattered *Phyllachora* stromata. Score 3 denotes a plant with a moderately resistant reaction, with 26–50% of the leaf area affected by a moderate density of chlorotic lesions and well-developed tar spot symptoms; score 4 denotes a plant with a susceptible reaction, with 51–75% of the leaves exhibiting large clustered chlorotic and necrotic spots; and score 5 denotes a plant with a susceptible reaction, with 76–100% of the leaves exhibiting large clustered chlorotic and often premature aging of the stems. The percentage numbers of disease severity can be calculated using the Horsfall & Barratt formula (1945) using the score data from the list above. Scoring data is not displayed in this report.

$$DS = \frac{\sum(Ni.Vi)}{N.V} \times 100\%$$

DS = Disease Severity (%);
Ni = Numbers of plants with i-scored;
Vi = The i-scored;
N = Number of the observed plants;
V = The highest score.

Morphological Identification. Morphology identification, both of macroscopic and microscopic detection, used the tar spot on the sorghum leaf, which was directly taken from the field. Macroscopic detection was carried out by direct observation of the pathogen, including the shape and size of the spots, the color of the spots and other symptoms around the spots. For the characteristics of macroscopic observation were spots, such as color, shape, size, and type of spot or colony. For the characteristics of microscopic was fruiting bodies of *Phyllachora*. Microscopic observations were started by sterilizing the leaf surface with tar spot symptoms. The leaf surface was gently wiped using a cotton swab moistened with 70% alcohol and then wiped with a cotton swab moistened with sterile water. A drop of water was put on the fruiting bodies and the fruiting bodies were removed from sorghum leaves using the tip of a scalpel and put onto an object glass ready for microscopic observation. Asci and ascospores characters were observed using a NIKON Eyepiece AM7025B microscope. Lactophenol blue was used as a dye. The characteristics of *Phyllachora* sp. were identified according to the references of Dayarathne et al. (2017) and Li et al. (2019).

RESULTS AND DISCUSSION

Sorghum is a cereal crop of the Poaceae family that has the potential to be developed in Indonesia with many advantages, including as a source of carbohydrates. It has begun to be widely cultivated in Indonesia, especially on marginal lands (Tabri & Zubachtiodin, 2013). One of the threats to sorghum cultivation in Indonesia is a disease, and most commonly, a fungal infection. Since 2018, the Indonesian government, through the ministry of agriculture, requires the freedom of any disease if the breeders want to release new varieties. It's a challenge for the sorghum breeder to develop sorghum for any purpose with disease resistance. Tar spot is one of the common diseases in sorghum, but there has been no report about it.

Tar spot is a disease that commonly attacks cereal crops, including sorghum. Tar spot disease was caused by *Phyllachora* infection. In maize, it was first detected in Illinois and Indiana, USA in 2015 and has been reported and confirmed by Mottaleb et al. (2019). Monteiro et al. (2003) stated that this pathogen in maize has a small damaging effect on the host, but if it is abundant on the leaf surface, it will have a huge effect because it blocks the sunlight entrance and inhibits the photosynthesis process. High yield losses due to this disease in maize can occur if the climate and weather conditions are favorable for the development of this pathogen (Cao et al., 2017). This research reported the incidence and severity of tar spot disease caused by *Phyllachora* infection on sorghum in three regions in Indonesia.

Tar Spot Disease of Sorghum Plants. The incidence of the tar spot disease in all varieties from three areas of sorghum cultivation in this report was 100%. The symptoms were recognized as shiny black spots like tar drops (Figure 1). The *Phyllachora* sp. formed colonies on the leaf surface with small black spots. The number of tar spot symptoms can be used as a parameter for the presence of the previous infection of the tar spot disease pathogen in those sorghum cultivation areas. The sorghum cultivation area in Gunung Kidul was previously cultivated side by side with corn and eucalyptus plants. The sorghum cultivation area in West Lombok is an ex-situ collection area of genetic resources belonging to the Indonesian Agency for Agricultural Research and Development, West Nusa Tenggara (BPTP NTB), which was previously planted with local corn. Based on this information, it is surely shown that the *Phyllachora* inoculum already existed in the field before.

Sorghum cultivation in the Bogor area has the same history as the two previous areas. The area was close to the sorghum cultivation area with tar spot disease. This area was the tar spot disease research area where tar spot disease artificial inoculation was carried out on the crops. Phyllachorales is an obligate fungal parasite. Mottaleb et al. (2019) revealed that *Phyllachora maydis* causing tar spot disease in maize, can survive on dry leaves and also on other parts of plants, such as the husks of maize ears etc. In plants with a high attack of tar spot disease, we also found the fruiting body of *Phyllachora* on leaf midribs and husks or seed coats (Kleczewski et al., 2020) as well

Figure 1. Tar spot of sorghum plants *(Sorghum bicolor)* caused by *Phyllachora* sp.

as on stems. Fruiting bodies on the leaves and parts of this plant are the source of inoculum for the next plantation, especially sorghum, which has a close relationship with maize.

Ruhl et al. (2016) stated that *Phyllachora maydis*, the pathogen of tar spot on corn, might have been introduced by air movement through the nearby area. The transmission is also because of the weather, which is warm and humid. Sorghum cultivation in Gunung Kidul was cultivated at the end of the dry season with no rain for 3 months, but in the morning it was often filled with mist that carried a lot of water vapour, so the conditions became humid and good for the tar spot disease spread and development. Similar to the sorghum cultivation in the Bogor area, it was cultivated at the end of the dry season, but with the weather anomaly, the conditions at that time were often rainy with slight to moderate intensity, so those conditions were good for the spread and development of disease. The severity of the tar spot disease on 9 varieties planted in Bogor, 2 varieties planted in Gunung Kidul and 6 varieties planted in West Lombok can be seen in Table 1.

The percentage numbers of disease severity can be calculated using the scoring data and are not displayed in this report. The severity of tar spot disease varied from 32.4% in the Latu Keta sorghum variety to the highest of 87.9% in the Samurai sorghum variety. From these numbers, we can conclude that the Latu Keta sorghum variety has much greater resistance to tar spot disease than the Samurai variety, which is susceptible to tar spot disease. The percentage number 32.4% in the Letu Keta variety according to Singh et al. (2022) from the Maximum Disease Severities (MDS) for tar spot is susceptible (30.0–48.3% MDS), which means medium severity of tar spot. According to the MDS number, the percentage number of 87.9% in Samurai variety is the most susceptible (more than 48.3% MDS) or high severity of tar spot.

The severity of tar spot disease on these 17 varieties of sorghum can be used as a reference in selecting resistant sorghum varieties to tar spot disease for sorghum cultivation. It can also be used as a reference to decide which varieties can be used as a genetic source for the breeding approach. The severity of tar spot disease in this study varied due to different varieties of sorghum because plants with different levels of disease resistance have different resistance mechanisms (Ahn et al., 2019).

Plants have a defense mechanism against pathogens or pests with inbred defense mechanisms or natural barriers both morphologically, physiologically, and biochemically (Das, 2019). The plant's defense mechanism will be shown after the plant is infected by the pathogen. The general defense mechanism is to minimize the invasion or reduce the development or action of the pathogen.

Sorghum produces phytoalexin, a defense molecule as a response to fungal infections. The phytoalexin compound interacts with the apressoria of the fungi and kills the pathogenic fungi (Das & Rajendrakumar, 2017). Sorghum's defense mechanism against pathogenic fungi that cause leaf spots is generally related to cell walls, such as the accumulation of reactive oxygen species (ROS). ROS accumulation is activated during the biotrophic phase to prevent the development of pathogenic fungi (Puttalingaiah, 2011). Sorghum's other defense mechanism against

Table 1. Numbers of tar spot disease severity of sorghum plants from three district in Indonesia

District	Sorghum Varieties	Disease Severity (%)
Bogor	Konawe Selatan	44.0
	4183	47.2
	Sorgum Malai Mekar	50.0
	Kawali	53.1
	Super 1	53.6
	181	53.5
	Jagung Rote	55.9
	JP	63.0
	Pahat	72.2
Gunung Kidul	Samurai	87.9
	Bioguma	77.4
West Lombok	Latu Monca	62.0
	Latu Kaca	71.6
	Gondo Keta	69.2
	Gondo Bura	78.0
	Latu Keta	32.4
	Latu Keta Na'e	32.8

pathogenic fungi is lignification (Bhuiyan et al., 2009). During the defense response, lignin phenolic compounds or accumulated lignin-like compounds can prevent pathogen infection. Another defense mechanism related to lignin compounds in plants as a response to fungal infection is the hypersensitive response (HR) associated with programmed cell death (PCD). Lee et al. (2019) elaborated on the role of lignin in disease defense mechanisms by preventing HR-PCD. Cell death becomes a barrier for the pathogen. Pathogens cannot invade healthy cells in the absence of nutrients because they are blocked by the barrier. The defense mechanisms of sorghum against fungal pathogen infection, especially the fungus that causes tar spot, should be carried out to obtain useful information for breeding and developing disease-resistant sorghum plants.

Morphologi Identification. Morphological identification was carried out both macroscopically and microscopically. The macroscopic observation results of the symptoms caused by this fungus on the sorghum leaves indicate that this fungus is a fungus in the genus *Phyllachora*, family *Phyllachorales*. This was as stated in the taxonomic section of several *Phyllachora* species that infect several Poaceae plants in an Dayarathne et al. (2017). The macroscopic observations results about *Phyllachora* parasitized sorghum in this study were similar to Dayarathne report about *Phyllachora* parasitized Poacea plants. The symptoms from this tar spot on the sorghum leaves were characterized by black spots on the leaf surface with a size of 1-2 × 1-3 mm, the shape of the spots is round and as the disease progresses it is mostly oval and elongated. The shape of the spots is raised and shiny. The number of spots increases through the disease progresses and it depends on the resistances of the host, gregarious and scatteres and surrounded by a yellow halo of discolored leaf tissue. The further infection will cause these spots to enlarge and will spread to other leaves and finally to all leaves on the plant (Mahuku et al., 2013).

The microscopic observations resulting from tar spots on the sorghum leaves (Figure 2) were: paraphyses 0.93–2.27 µm wide, filiform and numerous, unbranched, septate, longer than the asci and tapering apices. Asci measure 62.3–74.7 × 12.0–15.3 µm ($\bar{x}$ = 68.9 × 13.4 µm, n=30), 8-spored, unitunicate, long, cylindrical to fusiform, obtuse to rounded apex, and uniformly thick walls. Ascospores 19.8–13.0 × 5.0–6.3 µm ($\bar{x}$ = 11.5 × 5.5 µm, n= 30), uniseriate with sometimes overlapping and oblique, hyaline, constrictions at the centre), some are ellipsoidal and occasionally ovoid, aseptate with one-celled. These microscopic observations of ascospores (Figure 2.F–2.H), asci (Figure 2.I–2.K), and paraphyses (Figure 2.E) were similar to other microscopic types of *Phyllachora* that infected other

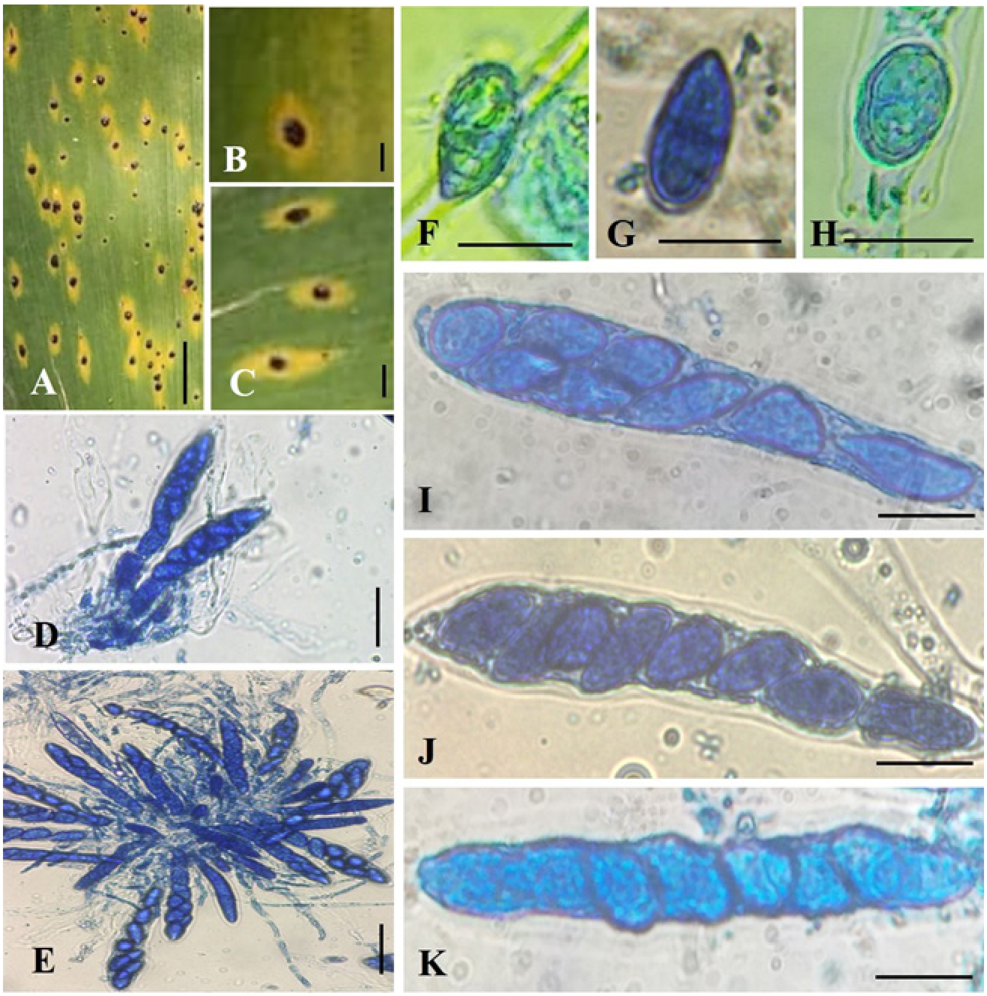

Figure 2. *Phyllachora* sp. that infected sorgum leaves caused of tar spot disease. A. Tar spots on sorghum leaves; B–C. Close up of tar spots; D–E. Asci and paraphyses; F–H. Ascospores; I–K. Asci. Scale bars: A= 10 mm; B–C= 1 mm, D–E= 20 µm; F–K= 10 µm.

plants, such as Poaceae plants (Dayarathne et al., 2017), and infected *Cardiospermum grandiflorum*, also known as balloon vine or hearth pie plants (Pereira et al., 2010). From these similarities, we can conclude that the *Phyllachora* that caused tar spots on sorghum are the same *Phyllachora* that caused tar spots on other plants. Once again, this was the first report of tar spot on sorghum caused by *Phyllachora* sp., so molecular identification was needed to confirm the species of *Phyllachora* that infected sorghum.

CONCLUSION

Tar spot is a disease that commonly attacks cereal crops, including sorghum. The observation results of the tar spot disease incidence in 17 varieties of sorghum from Bogor, Gunung Kidul, and West Lombok were 100%. The severity of tar spot disease varied from 32.4% in the Latu Keta sorghum variety to the highest of 87.9% in the Samurai sorghum variety. From these values, we could conclude that the Latu Keta sorghum variety had much greater resistance to tar spot disease than the other varieties with medium severity of tar spot. The level of tar spot disease severity of these 17 varieties of sorghum could be used as a reference to decide which varieties could be used as a genetic source for the breeding approach. The severity of tar spot disease in this study varied due to different sorghum varieties. The macroscopic and microscopic observation results of tar spot on sorghum plants showed that the cause of the tar spot disease was *Phyllachora*. This was the first report of a tar spot on sorghum caused by *Phyllachora* sp. Therefore, molecular identification is needed to confirm the species of *Phyllachora*.

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AUTHORS' CONTRIBUTIONS

SW and SHH considered and planned the experiment. T and SN informed the sorghum plant damage area and supported the genetic resources. SN supported the microscopic observation. DA collected data on the plant damage area, performed analysis, and interpreted the data. DA, SW, and SHH prepared the manuscript. The authors provided responses and comments on the research flow, data analysis and interpretation, as well as the shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

There is no competing interest regarding this publication.

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