



Seed-Borne Fungi Associated with Foxtail Millet (*Setaria italica* L. Beauv.) Genotype ICERI-6

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ABSTRACT

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The need of functional foods that provide health benefits beyond the essential nutrient has caused foxtail millet (*Setaria italica* L. Beauv.) potential to develop. The foxtail millet superior development needs to be equipped by the production of pathogen-free seeds. Seed-borne pathogens have the potential to inhibit plant growth, reduce plant productivity, change the nutritional content of plants, and may cause new plant disease epidemics. This study aimed to detect and identify fungi associated with foxtail millet seeds genotype ICERI-6 from the Indonesian Cereal Research Institute, Maros which have 8 mo seed storage period. Seeds surface-sterilized with NaOCl was grown on potato dextrose agar (PDA) and incubated for 4 days. Detected fungal colonies were recultured on PDA medium to be characterized by morphological characteristics. The colonies were dominated by *Fusarium oxysporum* (52%) followed by *F. verticilloides*, *Curvularia* sp., *Helminthosporium* sp., *Cladosporium* sp., and *Rhizoctonia solani* ranging from 4% to 13%. The fungal growth rate varies from 0.73 - 2.67 cm per day. Through pathogenicity test, all of the isolates are pathogenic. Hot water treatment with temperature 52 °C for 20 min could reduce the percentage of infection by up to 64% without the reduction of seed germination. Detection of pathogens at different seed storage period and genotypes is needed as basic information to optimizing the method of controlling seed-borne pathogen in foxtail millet seeds.

INTRODUCTION

Foxtail millet (*Setaria italica* L. Beauv.) is a functional food that has high potential to be developed in future plant breeding. This plant has several superiorities such as high protein, antioxidant, fiber, mineral, and supplementation to control type-2 diabetics (Thatola 2010). Moreover, this millet is tolerant to water and salinity stress (Moharil et al. 2019). These agronomical superiorities have to be followed by an effort to produce healthy seeds. Healthy

seeds will produce plants with optimum productivity with nutritional stability as well as its genetic potential. Also, healthy seeds can prevent or minimize disease incidence in the field due to the transmission of seed-borne pathogens.

Seed-borne pathogen dominated by fungi and its transmission leads seed abortion, seedling growth inhibition, disease outbreak, and toxic compound production (Kavitha and Vijayalakshmi 2011). The symptoms of seed-borne fungi infection are not always present

thus visual detection is not sufficient to determine the seed health status. Common seed-borne fungi infected Poaceae family are *Helminthosporium* spp., *Alternaria alternata*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp., *Mucor* spp., and *Rhizopus* spp. The seed storage period is also affecting the population, diversity, and domination of seed-borne fungi which lead to different seed treatments. However, the information about seed-borne pathogen in foxtail millet seed in Indonesia has not been reported. Detection and identification of seed-borne fungi from genotype ICERI-6 with 8 months-old-seeds from this research will become basic information in pathogens elimination by modifying several controlling strategies.

MATERIALS AND METHODS

Foxtail millet seed used in this research was genotype ICERI-6 seeds from Indonesian Cereal Research Institute. This genotype is desirable due to its short stature, short harvest period, and relative tolerance to salinity and drought stress (Ardie et al. 2015, Lapuimakuni et al. 2018). The seeds used do not have visual pathogenic symptoms on its surface. Isolation of seed-borne fungi in this research used the incubation method using potato dextrose agar (PDA) added by chloramphenicol. Surface sterilization of these seeds used sterilized water (10'), followed by NaOCl 1% (10'), and rinsed 3 times with sterilized water (1'). Then, the seeds were dried on a sterilized tissue paper (5') to prevent the air microbes contamination. Seeds were placed in petri dishes containing PDA medium with 5 seeds in each unit and incubated in a temperature room for 4 days.

Each of the isolated fungal colonies was recultured in PDA medium to be characterized morphologically. The variables observed in this characterization were the color, growth pattern, growth rate, and diversity of the colonies. Olympus CH30 compound microscope was used for the observation of microscopic structures. Genus or species identification based on macroscopic and microscopic characters were conducted by using *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured*

Fungi and Key to Species, Illustrated Genera of Imperfect Fungi, An Illustrated Manual on Identification of Some Seed-Borne Aspergilli, Fusaria, Penicillia, and Their Mycotoxins, and The Fusarium Laboratory Manual. The pathogenicity test of foxtail millet seed was carried out on PDA medium. As a preliminary controlling strategy, hot water treatment of 52 °C (20') is conducted to measure the infection suppression and confirmed through the growing -on test.

RESULTS AND DISCUSION

There were 25 fungus colonies isolated from the foxtail millet seeds. Each seed only infected by one colony. Based on the morphological characters, 11 isolates were recultured to be characterized at 7 days after incubation. Macroscopic characterization was done to the 11 colonies resulted 6 different isolates. Further characterization found that 6 isolates were from different genus and species based on the macroscopic (Figure 1 and Tables 1) confirmed by microscopic characterization (Figure 2).

The *Fusarium oxysporum* fungi could have white, violetish-white, yellow, or light brown colony colors according to Watanabe (2010). This fungus has hooked microconidia with 3 septate and has ellipsoid microconidia with no septate. However, *F. verticilloides* has pink to violet colony color. Microconidia of *F. verticilloides* produced in monophialide with "rabbit ear" (verticilliate) shape conidiophore and the macroconidia are often absent (Leslie and Summerell 2006). Most of the seeds infected by these two species of *Fusarium* could not be

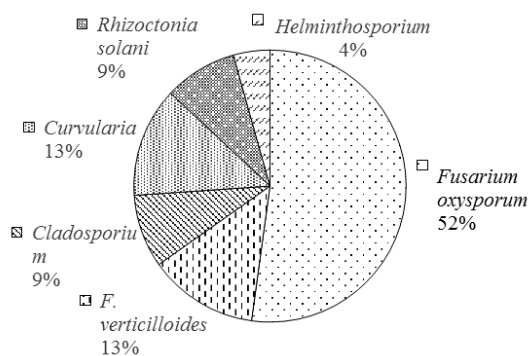


Figure 1. Domination and diversity of seed-borne fungi isolated from foxtail millet seed genotype ICERI-6

Table 1. Macroscopic characterization of six seed-borne fungi isolated from foxtail millet seeds

Isolates	Colony colour		Growth pattern
	Obverse	Reverse	
<i>Fusarium oxysporum</i>	White	White	Aerial
<i>Rhizoctonia solani</i>	Yellowish-white	Yellow	Aerial
<i>Helminthosporium</i> sp.	Brownish-grey	Black	Aerial
<i>Cladosporium</i> sp.	Dark grey	Black	Aerial
<i>Fusarium verticilloides</i>	Pink	Pinkish-violet	Aerial
<i>Curvularia</i> sp.	Grey	Black	Non-aerial

germinated. These fungi infected the embryo and endosperm of the seeds and also produced toxins (Watanabe 2010; Singh et al. 1991).

Barnett and Hunter (1998), Watanabe (2010), and Mirzaee et al. (2010) described the characteristic of other fungi. The fungus *Rhizoctonia solani* has 90°-shaped mycelia with the absence of conidia. This fungus will produce hard and round-shaped sclerotia at the end of the stationary phase. The conidia of *Helminthosporium* sp. has 4-5 septate with hylum on one of the conidial tip. However, *Cladosporium* sp. has a different shape of conidia. Round-shaped, oval, and irregular conidia with no septate could be produced in

Fusarium oxysporum

Rhizoctonia solani

Helminthosporium sp.

Cladosporium sp.

Fusarium verticilloides

Curvularia sp.

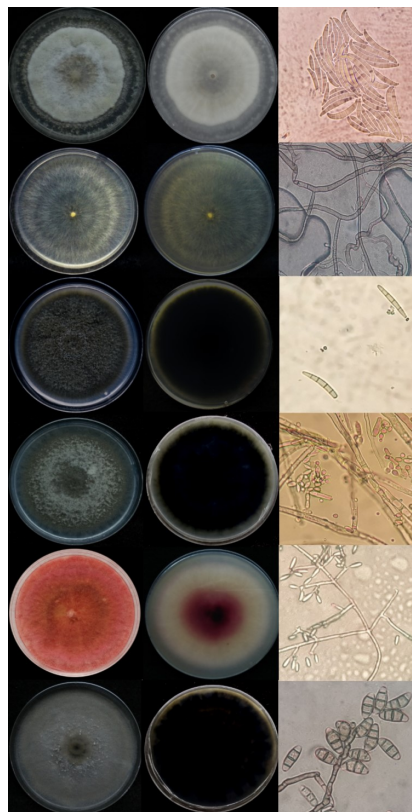


Fig 2. Colonies appearance of six fungal isolates (7 days) from foxtail millet seed; obverse (left), reverse (middle), microscopic structures (right) with 40x10 optical zoom.

one fungal colony. The distinction of *Curvularia* sp. characteristic is the dark and thick curved-conidia with 3-4 septate produced from long conidiophore. This fungus has an enlarged section in the middle of conidia. The seeds colonized by *R. solani*, *Helminthosporium* sp. and *Cladosporium* sp. could germinate normally but the seed colonized by *Curvularia* sp. have failed to germinate. The different effect from the fungi to the seeds depends on the different physiological activities such as the production of degradative enzymes, toxins, and other substances (Yago et al. 2011).

These colonies are dominated by *F. oxysporum* (Fig. 1) followed by *F. verticilloides*, *Curvularia* sp., *R. solani*, *Cladosporium* sp., and *Helminthosporium* sp. The domination of *Fusarium* spp. was also reported by Fard et al. (2014) who found 9 species of *Fusarium* from foxtail millet seed in Iran. The domination of *Fusarium* spp. was significantly related to its high pathogenicity as reported by Akanmu et al. (2013). However, this domination may depend on the genotype and seed storage period, thus the evaluation of the different genotype and seed storage period should be undertaken (Placinta et al. 2016). The growth rate of these fungi is also varying. Fungi *R. solani* has the highest growth rate (2.67 cm per day) followed by *F. oxysporum*, *Curvularia* sp., *Cladosporium* sp., *F. verticilloides*, and *Helminthosporium* sp. at 1.60, 1.60, 1.33, 1.14, and 0.73 cm per day, respectively.

Pathogenicity test showed that all of the isolates are pathogenic with symptoms such as necrotic in the roots, stems, and leaves and also damping-off. Related to the infection potential, elimination of seed-borne pathogens such as hot water treatment is needed to suppress the disease incidence (Elias et al. 2012). It has been observed in this research that hot

water treatment at 52 °C for 20 minutes could suppress the percentage of infection up to 64% by incubation method in PDA and growing-on test. This method need further research to optimized eliminate the seed-borne fungi up to 100% combined with other controlling methods. To our knowledge, this is the first report of seed-borne fungi associated with foxtail millet seed in Indonesia. Understanding the seed health status will expand the research scope especially to develop resistant varieties.

CONCLUSIONS

Six seed borne fungi associated with foxtail millet seeds genotype ICERI-6 are dominated by *Fusarium oxysporum* followed by *Rhizoctonia solani*, *Helminthosporium* sp., *Cladosporium* sp., *F. verticilloides*, and *Curvularia* sp. These fungi have a growth rate of 0.73-2.67 cm per day. All of the seed-borne fungi are determined as pathogenic fungi through pathogenicity test on PDA. Hot water treatment at 52 °C for 20 minutes could suppress the infection percentage by up to 64% confirmed by incubation method and growing-on test.

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