



Identification of Fungi Associated with Potato Tuber Causes Dry Rot in Lembang

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Abstract. Dry rot, caused by various species of *Fusarium*, is one of the most important fungal storage rots affecting potatoes. Infection of tubers occurs through wounds during planting, mechanical harvesting, and grading into size bands. The research aim was to identify the fungus carried by the tuber and its virulence that have been carried out at the Indonesian Vegetable Research Institute (IVegRI) Lembang, West Java from May 2020 until July 2021. Isolation and virulence tests were conducted at the Laboratory of Mycology at IVegRI. The observation variables were the morphological character of the fungus, Koch's Postulate test, and its virulence. In total, 8 isolates of *Fusarium* spp. were recovered and belonged to *Fusarium oxysporum* and *Fusarium* sp. In Koch's Postulate test, all isolates showed necrotic symptoms (rot volume) with varying necrotic areas. The necrotic areas of each isolate were Spudy 2 (1.81 cm), AR08 (1.17 cm), Atlantik 3 (1.12 cm), Sangkuriang 2 (1.06 cm), Atlantik 7 (without symptoms, 0.71 cm), Spudy 3 (0.44 cm), Papita 1 (0.34 cm), Sangkuriang 1 (0.40 cm), and Control (0.34) respectively. From the necrotic area value, it can be seen that Spudy 2 isolate had the highest virulence. We have identified four *F. oxysporum* species from eight isolates and symptoms of dry rot found either in the healthy or the sick tuber.

Keywords: *Fusarium oxysporum* · *Fusarium solani* · Soilborne

1 Introduction

The potato was included as one of the fifth vital vegetables for Indonesian people. In 2019, Indonesian potato production achieved 1,314,654 t, with a harvested size area was 68,223 ha [1].

Spudy, Papita, AR 08, and Sangkuriang are potato varieties that have been released by the Indonesian Vegetable Research Institute (IVegRI). Meanwhile, Atlantik is a kind of potato raw material industry that has been existed in a market. Spudy, Papita dan AR 08 are kinds of industrial potato materials that are suitable as chips material. Spudy's excellence is high in production. At the same time, Papita's excellences are high in production and good in taste. High in production and resistance to *Phytophthora Infestans* are AR 08's excellences [2]. Sangkuriang variety is more suitable for a vegetable dish, resistant to *Phytophthora infestans* and high in production. The main disease on potato in

field is leaf blight, and in storage is dry rot. There are a lot of studies regarding blight leaf caused *P.infestan* in Indonesia, but there is only few information about dry rot because *Fusarium* on potatoes tuber.

Dry rot is one of the diseases that attack potato tuber, caused by *Fusarium*. The disease is commonly found in potato centre production areas. The dry root attacking potato tuber is happened at the storage, after harvested. It could decrease a potato tuber yield by 60% [3, 4]. Also, it affects potato nutrition because of mycotoxins accumulation [5] that negatively influences the human body [4]. Potato tuber dry rot is happened because of an inappropriate harvest [5]. The physical tuber appearance attacked by dry root is brown patches to black, dried and lead the tuber wrinkled [6, 7]. The objective of study was to identify kinds of fungus that associate with potato dry rot disease.

2 Material and Methods

Materials used new harvesting potato tubers. The varieties were Spudy, AR08, Atlantik, Sangkuriang and Papita, PDA media, kitchen towel, and a plastic container with a filter on the bottom. Besides that, other tools were used, such as a cutter, a 5 mm diameter cork borer, and a microscope.

2.1 Sampling and Isolation of Pure Fungal Cultures

The study used potato tubers harvested from Balitsa experimental field, Margahayu, Lembang- West Bandung, in May 2020 - until July 2021. The healthy tubers and dry rot tubers were selected, then they were isolated in a laboratory. The tubers were cleaned under the flowing water, and then they were air-dried. The tuber was sliced into a 1 cm size, and then the surface was sterilized by soaking it into sterile distilled water, ethanol 70% for two minutes. After that, it was washed off with distilled water then air-dried on the kitchen towel. A five potato tuber slices were planted in PDA and incubated for a week. The activity process was done in aseptic. The fungus colony would appear and purify.

2.2 Identification of Fungi Carried Away by Potato Tuber

Purifying fungi isolate was identified under a microscope. A glass object was drizzling with sterile distilled water. Next, fungus colonies were taken using ose needle and were homogenous. The objects of observation were spore form, chlamydospore, and the hypha. Identification of fungi was used The *Fusarium* laboratory manual book [8].

2.3 Postulate Koch's Assay

Postulate Koch was tested using agar plug metode [9, 10] with slight modification. Sample tubers for testing used the newly harvested potato at Margahayu experimental field, Lembang. The tubers were cleaned in water. Then they were sterilized by soaking them in Natrium Hypochlorite 10% for 10 min. Then they were rinsed three times with sterile distilled water and air-dried on the kitchen towel for a night before inoculating.

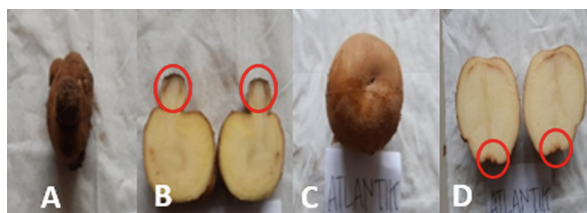


Fig. 1. Dry rot symptoms in the field (A), Symptom inside the tuber (B), The healthy tuber outward appearance (C), Dry rot symptom inside of healthy tuber outward appearance (D).

Tubers were perforated with a cork borer. About a 0.6 cm depth hole was made for each tube; then, the purified fungi isolate taken with a cork borer and inserted into the tuber hole. A tuber that had inoculated was put in a plastic container that had a filter on the bottom. Water sparingly was poured at the container base to keep its humidity. Each treatment consisted of 3 replications. The mycelium-inoculated tubers were incubated for two weeks at room temperature. Following incubation, potato tubers were cut from the point of inoculation (longitudinally) and the depth of internal necrosis was measured using a ruler. The depth of wound response in controls was also recorded for comparison. Mean rot volume was calculated used the formula: $V = 1/3 hr^2 \pi$, where h is the lesion depth and r is the radius [9].

3 Results and Discussions

3.1 The Disease's Symptoms from the Field

Symptoms of dry rot on the tuber made its surface have black patches and wrinkles (Fig. 1). If it pressed, the tuber was soft. When the tuber was sliced, the flesh colour was dark brown to black. A healthy tuber was also sliced to observe its flesh. It found a tuber with a good outward appearance. Nonetheless, there was brown necrotic inside. Minor symptoms were found on the tuber because it had just been harvested. However, if the tuber was stored longer, it predicted the symptoms would be evolved. The potato dry rot attacking in storage stage could reduce its yield up to 60% [11].

3.2 Fungal Isolates Carried Out by Potato Tuber

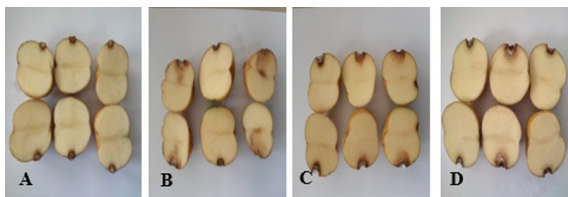
The study resulted 8 isolates, they were Spudy 2, AR08, Atlantik 3, Sangkuriang 2, Atlantik 7 (without symptoms), Spudy 3, Papita 1, and Sangkuriang 1.

3.3 Postulate Koch's Assay

The result of postulates Koch's showed that dry rot symptoms have appeared on the isolates of Spudy 2, AR08, Atlantik 3, Sangkuriang 2, Atlantik 7 (without symptoms), Spudy 3, and Papita 1. Meanwhile, Papita 1 did not show the sign. The symptom of inside tuber showed by brown colour to black and spread. The symptom area had a different symptom volume (Table 1). The distinction symptom volume at the same time

Table 1. Rot volume as measured by mean depth of necrosis (in cm) by Koch's postulate

No	Varieties	Rot volume
1	AR08	1.17
2	Spudy 2	1.81
3	Sangkuriang 1	0.40
4	Papita 1	0.34
5	Sangkuriang 2	1.06
6	Spudy 3	0.44
7	Atlantik 3	1.12
8	Atlantik 7 (without symptom)	0.71
9	Control	0.34

**Fig. 2.** Potato tuber rot volume in Koch's postulate, Control (A), Spudy 2 (B), AR08 (C), and Atlantik 3 (D).

showed about the isolate pathogenicity. Every *Fusarium* spp fungi pathogenicity is very different. Specifically for the potato, it is pathogenic to potato tuber dry rot and could become opportunistic [12].

In the Fig. 1, we only show three potato tubers with the largest volume of dry rot symptoms. From the Fig. 2, the biggest rot volume was Spudy 2 isolate (1.7 cm), following by AR08 isolate (1.17 cm), Atlantik 3 (1.12 cm), Sangkuriang 2 (1.06 cm), Atlantik 7 (without symptom) (0.71 cm), Spudy 3 (0.44 cm), Sangkuriang 1 (0.4 cm), and the Papita 1 (0.34 cm) same with the control. So, the Papita 1 isolate did not show the symptom. Time incubation was limited to two weeks; therefore, the dry rot symptom was mild. When the time of incubation is longer, the symptom would be evolved.

3.4 Identification of Isolates

Based on microscopic observation, it found *F.oxysporum* with 4 isolates dan *Fusarium* spp 4 isolates (Table 2). The colour of *F.oxysporum* was white, pale orange, pale violet, and beige. Leslie and Summerell (2006) describes that colony morphology on PDA of *F.oxysporum* widely varied. Mycelia may be floccose, sparse or abundant and range in color from white to pale violet. Macroconidia usually 3-septate, short to medium length, straight to slightly curved, relatively slender and thin walled. Morphology of apical cell was tapered and curved, sometimes with a slight hook (Fig. 3A). The abundance

Table 2. Character macroscopic and microscopic of fungi isolates

No	Varietas	Macroscopic (colour of colony on PDA)	Microscopic				Identified
			<i>Mycelia</i>	<i>Microconidia</i>	<i>Macroconidia</i>	<i>Chlamydo spores</i>	
1	AR08 2	white-pale violet	septate and branched	0 - 1 septate	absent	absent	Fusarium sp
2	Spudy 2	white-pale orange	septate and branched	0 septate	absent	absent	Fusarium sp
3	Sangkuriang 1	White	septate and branched	0 - 1 septate	4 cell	present	F. oxysporum
4	Papita 1	white-pale orange	septate and branched	0 - 1 septate	4 cell	present	F. oxysporum
5	Sangkuriang 2	White-pale yellow	septate and branched	0 - 1 septate	absent	absent	Fusarium sp
6	Spudy 3	white-pale violet	septate and branched	0 septate	4 cell	present	F. oxysporum
7	Atlantik 3	white-beige	septate and branched	0 - 1 septate	absent	absent	Fusarium sp
8	Atlantik 7 (without symptom)	white-beige	septate and branched	0 - 1 septate	4 cell	present	F. oxysporum

of macroconidia is sparse in some strains, but usually abundant in sporodochia and occasionally from hyphae growing on the agar surface. Microconidia shaped is oval, elliptical or kidney shaped and usually 0-septate and abundant in the aerial mycelia. Chlamydo spores is formed abundantly but some isolates form chlamydo spores slowly if they form them at all. Usually formed singly or in pairs, but also may be found in clusters or in short chains. The species *F. oxysporum* was the most frequent [11].

Generally, there are 13 *Fusarium* species associated with potato tuber dry rot [13]. Later, the numbers are added, including *F. ciliatum*, *F. reticulatum*, *F. torulosum* and *F. verticillioides* [14–16]. Stefańczyk [11] reported that the isolates were pathogenic to potatoes in laboratory tests in Poland were found in four species: *F. avenaceum*, *F. sambucinum*, *F. graminearum*, and *F. culmorum*.

Besides reducing yield, the potato tuber dry root also produces mycotoxins. It cause cyto-, geno-, neuro- and hepatotoxic effects in humans and animals and threatens their health when consumed directly or indirectly. Kinds of ultimate mycotoxins generated by *Fusarium* spp. are moniliformin, beauvericin, trichothecenes, zearalenone and fumonisins. Specifically for trichothecenes and zearalenone, these mycotoxins get attention as they cases often were happened. Trichothecenes are produced by *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. poae*, *F. sporotrichioides*, *F. langsethiae*, and *F. sambucinum* [17].

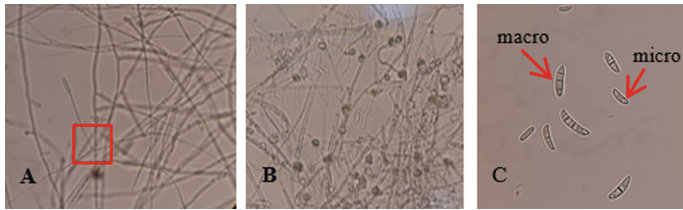


Fig. 3. Microscope character of *F. Oxysporum*, apical cell a slight hook (A), chlamydospores on mycelia (B), and macroconidia and microconidia (C).

Meanwhile, zearalenone is produced by *F. culmorum*, *F. equiseti*, *F. graminearum*, and *F. cerealis* [18]. However, the production of the mycotoxin are depended on environmental conditions [19], and the resistance of potato variety toward the fungi [20].

Our results showed the symptoms of dry rot found either in the healthy or the sick tuber. Many species from the *Fusarium* genus caused dry rot, and we have identified four *F. oxysporum* species from eight isolates.

4 Conclusion

Our results showed dry rot symptoms were found in both healthy and diseased tubers and we identified four species of *F. oxysporum* from eight isolates.

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