Biology Research in Thailand

Microscopic Observation and Pathogenicity Determination of Common Molds on Postharvest Longan Fruit cv. Daw

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Abstract

Longan (*Dimocarpus longan* Lour. cv. Daw) is one of Thailand's most important export fruits. This crop was confronted with a severe postharvest fungal rot disease problem. Examination of the surface appearance using a stereo-microscope showed that the fruit skin was rough and uneven. Under a scanning electron microscope, the surface of longan fruit consisted of scale and epidermal hairs, and in some areas the remnant of cuticle could be observed. Filamentous fungi were also observed. Many genera of fungi were isolated from the affected skin of harvested longans by a tissue transplanting method. These were *Aspergillus, Cladosporium, Colletotrichum, Fusarium, Lasiodiplodia, Mucor, Penicillium Pestalotiopsis, Phomopsis, Rhizopus, Trichoderma, Verticillium* and 7 unidentified genera. Each of the fungal isolates was inoculated onto the pericarp of the fruit for pathogenicity determination. The pathogenic ability examination showed that *Lasiodiplodia* and *Pestalotiopsis*, which were common molds found on the fruit skin, caused the most severe symptoms, e.g. the diseased fruit rotted rapidly. The most virulent isolate was identified as *Lasiodiplodia theobromae* based on its morphological characteristics and by DNA sequencing.

Key words: filamentous fungi, fruit rot, *Lasiodiplodia theobromae*, Longan, scanning electron microscope

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Introduction

A number of Thai researchers studied both pre- and postharvest disease of longan cv. Daw and found many genera of fungi in infected fruits, such as *Alternaria, Aspergillus*, *Botrytis*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Colleto-*

trichum, *Curvularia*, *Fusarium*, *Gloeosporium*, *Lasiodiplodia*, *Mucor*, *Nigrospora*, *Paecilomyces*, *Pestalotiopsis*, *Rhizoctonia*, and *Rhizopus* (Tongdee 1988, Coates *et al.* 2003). There is no published data on virulent isolates, which causes the most severe symptoms resulting the diseased fruit rotting rapidly.

Materials and Methods

Mature fruits of longan cv. Daw were harvested from a commercial orchard in Chiang Mai province. All fruits were transported to the laboratory within one hour after harvesting.

Anatomical Study

Pericarp surface anatomy was assessed using a stereomicroscope and a scanning electron microscope (SEM, JEOL JSM-5910LV). Tissue samples were obtained from several fruits, sectioned into 0.5 mm squares, and immersed into chilled 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2. The samples were washed with 0.1 M phosphate buffer pH 7.2, dehydrated using a graded acetone series and critical-point-dried. The tissues were mounted on copper stubs and spatter-coated with gold.

Isolation of Pathogenic Fungi

Fungi were isolated from affected longan fruits. Tissue pieces from the edge of diseased peel or stem-end were surface sterilized in 1% sodium hypochlorite solution for 3-5 minutes and then placed on potato dextrose agar (PDA) plates. The plates were incubated at room temperature and observed on a regular basis. The growing edges of any fungal colonies from the peel and stem-end pieces were then transferred aseptically to fresh PDA plates.

Pathogenicity Tests

All of the isolated fungi were tested for their pathogenicity on postharvest longan fruits. The pathogenicity was determined by dipping healthy fruits in the spore suspension of each fungus or by placing a mycelium disc on healthy fruits. The inoculated longan fruits were incubated in a foam tray. The virulent isolates, which caused the most severe symptoms, were selected and stored on PDA slants. For the control group, the fruits were dipped in sterilized distilled water and placed in a foam tray.

- *Preparation for inoculation*

Lasiodiplodia (a virulent isolate) was cultured on PDA plates. Inoculums were prepared in the form of mycelia discs of 0.5 cm in diameter.

- *Fruit inoculation*

Prior to inoculation, the fruits were dipped into 70% (v/v) ethanol to reduce the epiphytic microflora. A stem-end and an area of unwounded fruit peel were inoculated with the mycelia discs mentioned above. Fruits inoculated in the laboratory were placed on a foam tray and incubated at 25°C. For the control group, the same procedure was performed, except PDA discs (0.5 cm in diameter) were used instead of mycelia discs.

Identification of Lasiodiplodia to species

Morphological characteristics of *Lasiodiplodia* LP20 were studied under a light microscope and a SEM. Their mycelia were extracted for the DNA sequencing. The representative *Lasiodiplodia* sequences from the preliminary clades were used to obtain sequences from GenBank with a standard nucleotide-nucleotide BLAST search (Altschul *et al.* 1997).

Results

Anatomical structure

Study on the surface appearance using a stereomicroscope showed that the fruit skin was rough and uneven (Fig. 1a). Under a SEM, the surface of longan fruit consisted of scales and setae (Fig. 1b). In some areas remnants of cuticle could be observed (Fig. 1c). An abundance of microorganisms, *i.e.*, bacteria, yeasts and filamentous fungi, were observed on the fruit surface (Fig. 1c).

Isolation of pathogenic fungi

Two hundred filamentous fungi were isolated.

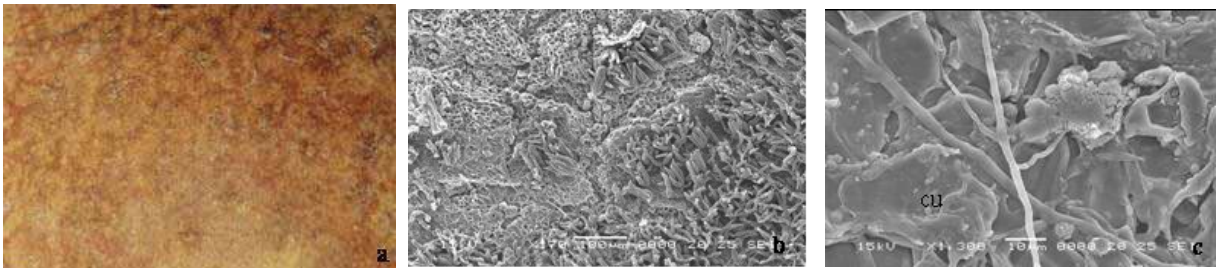

Figure 1 Stereomicrograph and scanning electron micrographs of longan fruit surface. a: The fruit skin was rough and uneven, b: It consisted of scales and setae (Bar = 100μm), c: Filamentous fungi were observed on the fruit surface (cu: cuticle; Bar = 10μm).

Table 1 The isolated fungi from the pericarp and stem-end of *Dimocarpus longan* Lour. cv. Daw fruits.

Taxa	Pericarp	Stem-end
Aspergillus LK1	+	+
Aspergillus LP2	+	-
Cladosporium CM	+	-
Collettotrichum LP1	+	-
Fusarium LP3	-	+
Lasiodiplodia LP20	+	+
Mucor CM	+	-
Penicillium LK5	+	-
Pestalotiopsis LYLP	+	+
Pestalotiopsis LK4S	-	+
Pestalotiopsis HCM20S1	-	+
Pestalotiopsis HCM23P1	+	-
Pestalotiopsis MLP	+	-
Phomopsis LK8	+	+
Rhizopus CM	+	-
Trichoderma LK9	-	+
Verticillium LK11	+	-
Unidentified LK1	+	-
Unidentified LK2	+	-
Unidentified LK10	+	-
Unidentified LK13	+	-
Unidentified LK15	+	-
Unidentified LK18	+	-
Unidentified LK19	+	-

They were identified to 19 genera. Some fungal isolates (Table 1) were selected and tested for pathogenicity.

Pathogenicity tests

Various molds grew well on longan fruits. However, only a few were able to cause necrotic lesions and rot the fruits (Table 2). Among these, *Lasiodiplodia* and *Pestalotiopsis*, were capable of causing the most severe symptoms and rotting the fruits rapidly. The most virulent isolate, *Lasiodiplodia* LP20, which was commonly isolated from the peel, was also indicated to be one of the most abundant pathogens of postharvest longan. Inoculated with this mold, the fruit was darkened (result of tissue necrosis) and then thoroughly decayed in 48 h at an ambient temperature (Fig 2).

Table 2 The pathogenicity of fungi isolated from *Dimocarpus longan* Lour. cv. Daw fruits

Genera	Inoculum	Disease Rating*
Aspergillus LK1	Conidia	0
Aspergillus LP2	Conidia	2
Cladosporium CM	Conidia	2
Collettotrichum LP1	Conidia	3
Fusarium LP3	Mycelia	2
Lasiodiplodia LP20	Mycelia	5
Mucor CM	Conidia	0
Penicillium LK5	Conidia	0
Pestalotiopsis LYLP	Conidia	4
Pestalotiopsis LK4S	Conidia	4
Pestalotiopsis HCM20S1	Conidia	3
Pestalotiopsis HCM23P1	Conidia	4
Pestalotiopsis MLP	Conidia	4
Phomopsis LK8	Mycelia	2
Rhizopus CM	Conidia	0
Trichoderma LK9	Conidia	2
Verticillium LK11	Conidia	0
Unidentified LK1	Mycelia	0
Unidentified LK2	Mycelia	2
Unidentified LK10	Mycelia	0
Unidentified LK13	Mycelia	2
Unidentified LK15	Mycelia	0
Unidentified LK18	Mycelia	0
Unidentified LK19	Mycelia	0

*Disease rating means the degree of symptom which was observed on the fruit surface ranging from 0 to 5 48 hours after inoculation; 0 = no necrotic lesion, 5 = brown necrotic lesion of more than 2 cm in diameter.

Figure 2 Disease symptoms on harvested fruits 48 h after being inoculated with *L. theobromae* LP20. Top: Uninoculated (control) fruits on which surface PDA discs only were placed. Bottom: Inoculated fruits on which surface *L. theobromae* LP20 mycelium discs were placed.

Identification of Lasiodiplodia to species

LP20 grew well on PDA plates (The rate of increase in diameter was ~ 2-3 cm/d), but spored rarely on this medium. To induce sporulation, small pieces of sterilized grass leaf were scattered on the surface of the full-grown colony. The mold also sporulated well on potato dextrose broth (Fig. 3). Young spores were hyaline and one-celled. Ma-

ture spores were dark brown, thick walled and two-celled (Fig. 4). Inoculated peel observed under a SEM showed numerous hyphae (Fig. 5). Identification followed von Arx (1981) and Sutton (1980). By morphology studies, DNA sequencing and blast search, it came out that LP20 was *Lasiodiplodia theobromae*.

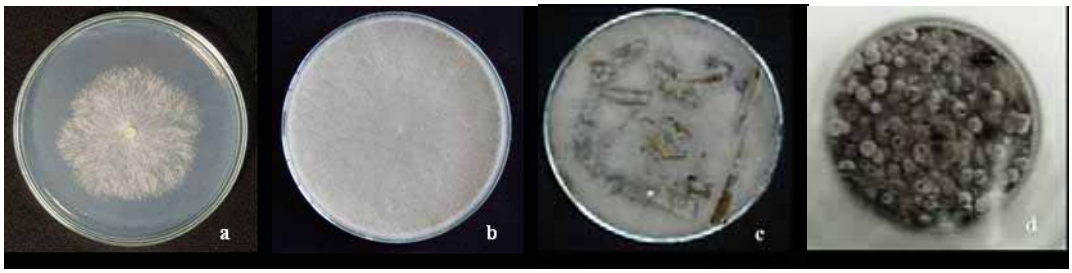

Figure 3 Colony growth of *L. theobromae* LP20. a) 1 day after inoculation on a potato dextrose agar (PDA) plate; b) 2 days after inoculation on a PDA plate; c) 2 days after placing pieces of sterile grass leaf on the culture of (b); d) 4 days after inoculation in potato dextrose broth

Figure 4 Light micrographs of *L. theobromae* LP20. a) Conidia, conidiogenous cells and paraphyses of a pycnidium; b) Immature, hyaline, aseptate conidia; c) A mature, dark brown, septate conidium (arrow).

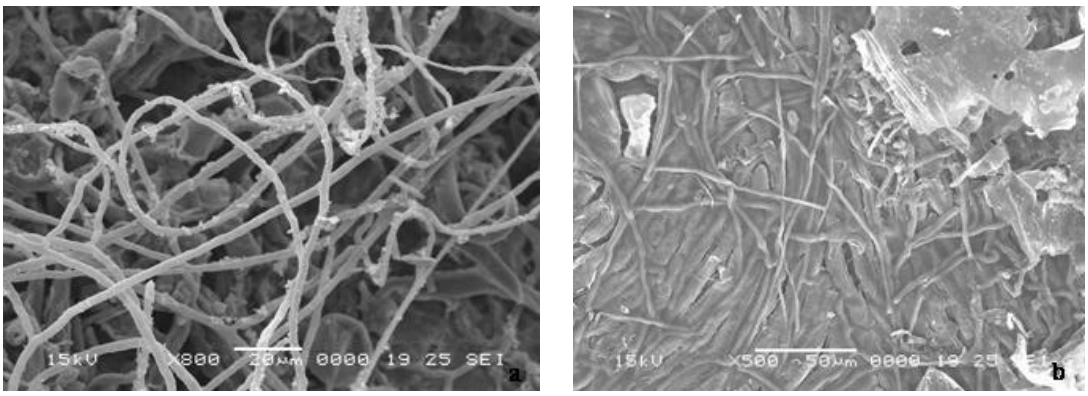

Figure 5 Scanning electron micrographs of *L. theobromae* LP20. a) hypha on the exocarp of longan fruit observed 24 h after inoculation (Scale bar = 20μm); b) hypha in the endocarp of longan fruit observed 24 h after inoculation (Scale bar = 50 μm).

Discussion

Under a light microscope, various microorganisms were found on the cuticular layer of the longan fruit surface. Many of them were hidden under epidermal hairs and in cicatrices. Isolation to pure culture by Suwanakood *et al* (2004) indicated that these microorganisms were yeasts, bacteria and molds.

The present research concentrated on molds because rot disease caused by mold was frequently reported to be the main problem of postharvest decay in longan fruits. In the present study, 19 genera of mold were isolated from longan fruit surface. Only two genera; *Pestalotiopsis* and *Lasiodiplodia* were commonly found on both healthy and infected fruits. These fungal genera were reported to be common pathogens for longan and some other fruits both pre- and postharvest (Pandey and Dwivedi 1987, Jonnson et *al*. 1994, Sangchote and Saoha 1998). They cause severe symptoms and rot the fruits rapidly. Sardsud *et al*. (1998) reported that *L. theobromae* and *Pestalotiopsis* were endophytic fungi of the longan tree. Suwanakood *et al*. (2004) found that *Pestalotiopsis* spp. were commonly found in healthy and rotten longan fruits.

As stated above, rot disease caused by mold is one of the most serious problems of exporting fresh longan fruits. Sulfur dioxide fumigation can completely stop the fruit decaying (Han *et al*. 1999, Tongdee 1994); however, its toxic residue remains a big problem (Udomchote 1994). The present study helped us to understand the main causal agents of longan fruit decay and the areas where they are hidden on the fruit surface. The study may possibly lead us to find alternative ways to control the pathogens.

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