Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Etiological agents of crown rot of organic bananas in Dominican Republic

Mohamed Abdalla Mohamed Kamel^{a,b}, Paolo Cortesi^a, Marco Saracchi^{a,*}

^a Università degli Studi di Milano, Department of Food, Environmental and Nutritional Sciences, Via Celoria 2, 20133 Milano, Italy ^b Plant Pathology Research Institute, Agricultural Research Center, 9 Gamaa St., 12619 Giza, Egypt

ARTICLE INFO

Article history: Received 10 February 2016 Received in revised form 31 May 2016 Accepted 2 June 2016 Available online 17 June 2016

Keywords: Colletotrichum musae Fusarium spp Lasiodiplodia theobromae Cavendish AAA Postharvest disease

ABSTRACT

Crown rot is a postharvest disease with a great negative impact on banana fruit quality. The infections occur at harvest, but the symptoms appear after overseas transportation. Different fungal pathogens are involved in crown rot, varying according to farming area. In this study we focused on etiology of organic banana crown rot in the Dominican Republic, which is one of the leading exporters of organic bananas. Bananas from five organic farms and their corresponding packing stations located in the Valverde province were studied. Over a period of three years, 558 banana hands were collected and a total of 5000 fungal colonies were obtained from the crown tissues and 518 representative colonies were purified, characterized and identified using morphological and molecular methods. Fungi were found in all samples from field and from packing house and were distributed in 11 genera. The fungal community was dominated by Fusarium, the most frequent genus (55%) found in more than 80% of all samples. The genus was represented by nine species; Fusarium incarnatum 53%, the most frequent, followed by Fusarium verticillioides 12%, Fusarium sacchari 12%, Fusarium proliferatum 7%, and Fusarium solani 6%. The five least frequent species were Colletotrichum musae, 7% overall frequency and found in 13% of all samples; Lasiodiplodia theobromae and Lasiodiplodia pseudotheobromae, 4% and 1% overall frequency, respectively, both species found in 7% of all samples; Nigrospora sp. 11% overall frequency, Alternaria spp. 6% overall frequency, followed by Phoma spp., Pestalotiopsis sp., Curvularia spp., Microdochium sp. and some other species, known to be saprophytes, with frequency lower than 2%. The etiological agents of crown rot disease can be ranked based on their presence and pathogenicity as follows: Fusarium incarnatum, Colletotrichum musae, Fusarium verticillioides, Fusarium sacchari, and Lasiodiplodia theobromae.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Banana is one of the most important world tropical crops cultivated in more than 100 countries (Arias et al., 2003), with a total production in 2012 of 133.3 million tonnes (FAOSTAT, 2015). Dominican Republic is the biggest exporter of organic banana to Europe (FAOSTAT, 2015).

Bananas are affected by several diseases, and crown rot is considered one of the most important postharvest diseases, causing a great negative impact on fruit quality. Fungi are the causal agents of crown rot, and they belong to different genera and species, whose presence and frequency varied according to farming area (Adjei, 2010; Alvindia et al., 2002, 2000; Alvindia

* Corresponding author.

E-mail addresses: dr_mohamed.kamel@yahoo.com (M.A.M. Kamel), paolo.cortesi@unimi.it (P. Cortesi), marco.saracchi@unimi.it (M. Saracchi).

http://dx.doi.org/10.1016/j.postharvbio.2016.06.002 0925-5214/© 2016 Elsevier B.V. All rights reserved. and Natsuaki, 2007; Anthony et al., 2004; Greene and Goos, 1963; Griffee, 1976; Griffee and Burden, 1976; Knight, 1982; Krauss and Johanson, 2000; Lassois et al., 2010; Reyes et al., 1998; Umana-Rojas and Garcia, 2011a; Wallbridge, 1981). Susceptibility of banana fruits to crown rot is influenced by seasonal variation determined by many pre-harvest factors (Ewane et al., 2013). Infections start at harvest and during packing (deBellaire and Mourichon, 1997) and infected flowers are the main inoculum source for Fusarium spp. and for Colletotrichum musae, where C. musae can cause both crown rot and anthracnose (Kamel et al., 2016), and some of these pathogenic fungi were also found on decaying leaves (Meredith, 1962). Additionally, fungal inoculum on banana stalks is knife-transferred onto the cut crown surface at dehanding (Finlay et al., 1992) or when clusters are cleaned in contaminated water (Shillingford, 1977). Crown rot symptoms appear after overseas transportation as blackening and mold of the crown area and this renders fruit unmarketable. To reduce crown rot severity, fungicides are widely used with the exception of







organic farming where use is forbidden (Eckert and Ogawa, 1985; Joas and Malisart, 2001). Therefore, for organic farmed bananas, there is the need to find appropriate tools for disease management based on disease etiology. Of the few scientific papers on crown rot disease of organic bananas, Umana-Rojas and Garcia (2011a,b) studied the cultivar "Gros Michel" organically cultivated in Costa Rica and found that the highest percentage of fungi isolated were F. subglutinans, Acremonium sp. and Colletotrichum musae, whereas F. verticillioides was only recovered from fruit grown under an integrated management system. Moreover, a study on "nonchemical" banana fruit and farms found that the common fungi imported on fruit into Japan from the Philippines, were: Acremonium strictum, Arthrinium phaeospermum, Aspergillus flavus, Colletotrichum musae, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium incarnatum, Fusarium oxysporum, Fusarium solani, Fusarium verticillioides, Gliocladium roseum, Glomerella cingulata, Lasiodiplodia theobromae, Phomopsis sp., Phyllosticta musarum, and Thielaviopsis paradoxa (Alvindia et al., 2002).

The research presented here focuses on identification of fungi associated with organic bananas of the cultivar "Cavendish" in the Dominican Republic, at various handling steps, from field to packing house and on the assessment of their pathogenicity.

2. Materials and methods

Five banana (*Musa* AAA, Cavendish) plantations, covering approximately 750 ha, and their packing station, located in the Valverde province, Dominican Republic were investigated.

2.1. Sampling

Between February and March 2013, in each plantation, ten symptomatic banana hands were randomly sampled and delivered to the laboratory of Plant Pathology, Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy, for preliminary identification of pathogenic fungi.

The sampling for the identification of fungi associated with organic bananas was repeated over the season of three years, randomly collecting symptomless crowns in the field and at the packing station. The sampling covered all the steps of cultivation and post-harvest handling of bananas (Table 1). Flower and crown parts were randomly sampled at deflowering (flowers are removed 3 d before the developing bunches are sleeved) and at harvest. Hands of bananas were randomly sampled from each step of handling at the packinghouse: dehanding, delatexing, clustering and crown trimming, second washing, post-treatment (banana crowns are usually treated with disinfectant products) and then fruit packaging. Additional samples of cut crown debris were collected at clustering and crown trimming steps. Each hand was considered a separate sample and 3 samples were collected for each handling-step, and maintained in a paper bag. One-third of all

samples were analyzed in the Dominican Republic, and the remaining two-thirds were shipped to Milan by airplane for further analysis.

2.2. Isolation

Fungi were isolated from the flowers and the crown. From the flowers, the basal part of female flowers attached to banana fingers was disinfected with sodium hypochlorite 5% for 120 s and subsequently rinsed in sterile water. Each sample was cut into five small pieces, placed to dry on sterile filter paper and then transferred into Petri dishes, 90 mm in diameter, on the surface of Potato Dextrose Agar, (PDA) (Difco Laboratories, USA) added with Nalidixic acid, Novobiocin, and Streptomycin sulfate, 25 mg L⁻¹ each, to inhibit bacterial growth. The crowns were surface disinfected as described above and after removing the outer layer, five pieces of about 5 mm² were sliced aseptically at increasing depths, and placed to dry on sterile filter paper under a sterile air flow. Subsequently, each sample was cut into 5 fragments and transferred on the surface of a Petri dish as described above. Plates were incubated for 6 d at 24 °C.

2.3. Purification and preservation

The plates were periodically observed using an optical microscope to count and identify the fungi grown on each sample. Representative colonies with the same phenotype were selected and purified on PDA, Malt Extract Agar (malt extract 20 g, agar 15 g, 20 g glucose, peptone 1 g, and water 1 L; pH 6.5 ± 0.5) (MEA), and Czapek Solution Agar (Difco Laboratories, USA) amended with 0.5% yeast extract (CYA). Single-spore isolation was carried out as described by Choi et al. (1999) with minor modifications. From sporulating mycelium, a loop of spores was transferred into a tube containing 10 mL of sterile distilled water and serially diluted to 10^{-8} . One milliliter of spore suspension was spread onto the surface of four PDA plates and then incubated overnight at 24 °C. A single germinated spore was transferred, with a sterilized needle, onto a new PDA plate. For non-sporulating mycelia, individual hyphal tips were excised under the microscope with a sterilized needle and transferred onto a new PDA. Pure cultures were stored at 4°C on PDA slants.

2.4. Identification based on morphological and cultural characters

Morphology and cultural characters of individual isolates were examined on PDA, MEA and CYA, following incubation at 24 °C for 7 d. Colony size, color and zonation were recorded for three plates of each isolate (Than et al., 2008). Growth rate was calculated on the relative increase of colony diameter. Mycelium morphology and reproductive structures were described and measured using an optical microscope Orthoplan (Leitz, Germany) equipped with a digital camera (Coolpix 4700, Nikon, Japan). Isolates sharing the

Table 1

Sampling areas and number of asymptomatic samples collected from each handling-process step.

Geographical coordinates of different farms	Cultivation area (Ha)	Sampling steps								
		Flowers	Crown parts from field	Dehanding	Delatexing bath	Clustering and trimming	Debris	Washing bath	After Alum treatment	Packaged fruits
19.615303, -71.090473	131.64	15	15	15	15	15	15	15	15	15
19.634726, -71.267431	48.43	7	7	7	7	7	7	7	7	7
19.657855, -71.304821	157.73	15	15	15	15	15	15	15	15	15
19.657057, -71.319509	299.32	15	15	15	15	15	15	15	15	15
19.642830, -71.352167	103.14	10	10	10	10	10	10	10	10	10

Download English Version:

https://daneshyari.com/en/article/4517733

Download Persian Version:

https://daneshyari.com/article/4517733

Daneshyari.com