DOI: 10.13140/RG.2.2.26063.69284
http://dx.doi.org/10.13140/RG.2.2.26063.69284
Cultivos Tropicales, 2016, vol. 37, no. 4, pp. 84-88 October-December

ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

Short communication
CONTAMINANT FUNGI IN *In Vitro* ESTABLISHMENT OF POTATO APEXES

Comunicación corta
Hongos contaminantes en el establecimiento *In Vitro* de ápices de papa

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ABSTRACT. Potato (*Solanum tuberosum* L.) is one of the most important food sources worldwide and it is affect for some pathogen either in the field or in the storage conditions. Some of the pathogens that attack this crop may remain in the tuber and they can be taken to the field at the time of planting so it is very important to obtain high quality potato seed for which *in vitro* culture is a useful technology. The aim of this work was the identification of fungal contaminants at the *in vitro* establishment phase of apexes of two potato variety (Fianna and Atlantic). Minitubers were storage in the dark at 18 °C for 20 days to obtain apexes that were collected, disinfected and placed in Murashige and Skoog medium supplemented with sacarose and agar. After seven days of apexes establishment, 100 % of the samples showed contamination and damage inside, which were identified in an optical microscopy as *Fusarium* and *Penicillium* genera, both tuber pathogen. This research demonstrate once again the importance to have healthy and high quality material for *in vitro* culture and the absence of sanity in the material sold to the farmers to obtain pre-basic seed or G1.

Key words: culture, Fusarium, Penicillium

RESUMEN. La papa (*Solanum tuberosum* L.) es una de las principales fuentes de alimentación a nivel mundial y es afectada por múltiples patógenos, ya sea en el campo o durante su conservación. Algunos de los patógenos que atacan a este cultivo pueden permanecer en el tubérculo y ser llevados al campo en el momento de la plantación, por lo que cada vez es más importante la obtención de semilla de papa de alta calidad fitosanitaria, donde el cultivo *in vitro* es una tecnología de gran utilidad. El objetivo del presente trabajo fue identificar contaminantes fúngicos en la fase de establecimiento *in vitro* de ápices de dos variedades de papa (Fianna y Atlantic). Los minitubérculos fueron almacenados en la oscuridad a una temperatura de 18 °C durante 20 días para la obtención de los ápices, los cuales se colectaron, desinfectaron y colocaron en un medio basal Murashige y Skoog suplementado con sacarosa y agar. A los siete días de establecerse los ápices, el 100 % de las muestras mostró contaminación y daños en el interior, identificándose en un microscopio óptico, los géneros fúngicos *Fusarium* y *Penicillium* como patógenos del tubérculo. Esta investigación apunta una vez más a la importancia de generar material sano de alta calidad para el cultivo *in vitro* y la falta de sanidad en el material que se les vende a los productores para obtener la semilla pre-básica o G1.

Palabras clave: cultivo, Fusarium, Penicillium

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INTRODUCTION

Potato (*S. tuberosum*) is one of the most important crops worldwide. In Mexico, Sinaloa and Sonora states are the main producers of this solanácea, reaching only established in Sinaloa 14,000 ha annually, representing 22 % of the National surface. The use of high quality material as starting (original seed), is a must for healthy and in good yields seed requirement; however, the acquisition of these is a problem for many producers middle- and low-income, mainly due to high costs.

One of the main systems used for the original seed is the *in vitro* multiplication or micropropagation for the subsequent obtaining minitubers free from pathogen (1-3). The culture media used in this system provide a rich blend of nutrients that can also allow rapid development of fungi and bacteria. Once these contaminants are established in culture medium, grow rapidly depleting nutrients from medium and producing harmful toxins for the explant (4).

For the above and considering that minitubers potatoes may contain endogenous pathogens (transmitted from generation to generation) and this is one of the first studies on the potato varieties that address the endogenous contamination of the plant material, the aim of this work was to identify the presence of fungal contaminants in the process of *in vitro* establishment of apexes of potato in the Fianna and Atlantic varieties.

MATERIALS AND METHODS

Plant Material and implementation of the apexes

From the state of Mexico, Fianna and Atlantic variety minitubers were placed in an incubator at 18 °C for 20 days to get the apexes. Subsequently carefully disinfected with running water and detergent, followed by three rinses with distilled water for five minutes, then they were washed for 15 minutes with 20 mL of distilled water (six drops of Tween 20) and rinsed again with distilled water to remove Tween residues. Immediately they immersed in 70 % ethanol for five minutes and washed with sterile distilled water. In laminar flow conditions, the apices were cut performed and placed in 2 % of sodium hypochlorite for 15 minutes. Later they were rinsed with sterile distilled water three times and allowed to dry for 15 min before placing them individually in tubes test containing basal Murashige and Skoog medium (5) with vitamins, supplemented with 20 g L^{-1} of sucrose and 4 g L^{-1} of solidifying gel (pH 5,8 ± 0,02). Incubation conditions were an eight-hour photoperiod light at a temperature of 25 ± 1 °C (6).

Identification of fungal contaminants through the optical microscope

Fungi developed in the apices cultured *in vitro*, they were isolated for their cultural and morphological identification in the culture medium potato-dextrose-agar and cultivated once, it was proceeded to microscopic observation on microscope slides.

The morphological characteristics of the reproductive structures of the fungus were visualized with an optical microscope Leica DM500 brand, with 40 x objective and a Leica ICC50 HD camera mounted thereon corresponding pictures were taken. Gender identification was performed by using keys and consulting specialized mycological literature (7). Furthermore, evaluation of percentage of explants for each fungus was performed by a percentage ratio based on the sample of 20 explants per variety.

Isolation and identification of contaminating fungi was performed at the Laboratory of Molecular Biology of phytopathogenic the National Polytechnic Institute, CIIDIR (Interdisciplinary Research Center for Regional Integral Development), Unit Sinaloa.

RESULTS AND DISCUSSION

100 % of contamination fungal apices potato grown *in vitro* was obtained, seven days after implantation in MS medium (Figure 1A), so it was decided to make cuts of minitubers with a scalpel dissection, being dry rot symptoms within the same for both varieties (Figure 1B and C).

The cultural and morphological identification of fungi isolated by light microscopy, resulted in the presence of *Fusarium* sp. and *Penicillium* sp. (Figure 2).

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Figure 1. Contamination by fungi of the apexes (A); Variety Atlantic minitubers (B) and Fianna Variety (C)

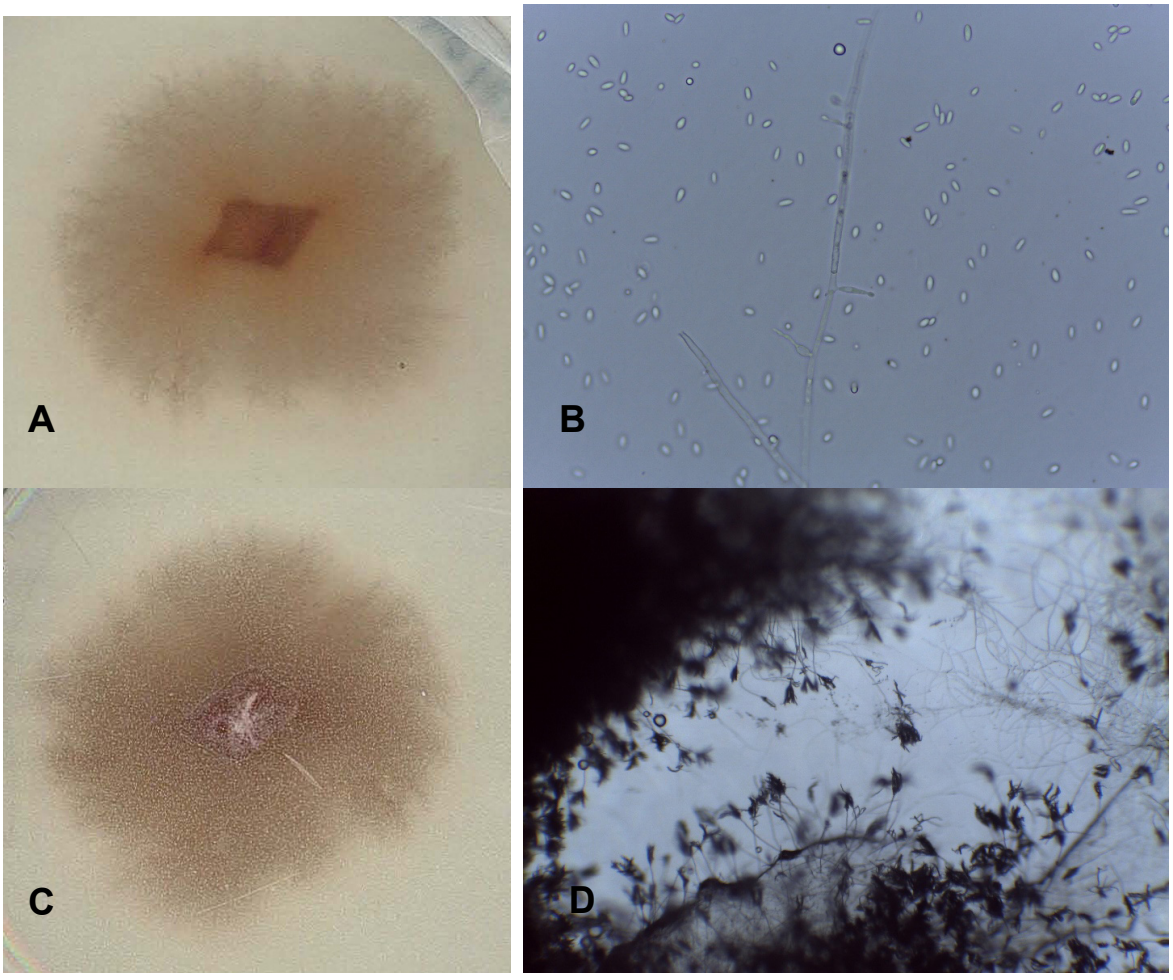

Figure 2. Morphological characteristics of isolates correspond with cottony mycelium pinkness (A) and microconidia of 7,238 x 2,96 microns (B) of *Fusarium* sp .; cottony slightly mycelium of green-blue hue tonilidad(C) and branched conidiophores and development of spherical conidia in chains 2,56 m in diameter (D) of *Penicillium* sp.

It is reported that there are more than 8,000 species of fungi that cause disease in plants (8), which are related to external and internal tissues thereof (9). Potato, *Fusarium* and *Penicillium* genera have been reported to cause disease (10). Infection for Fusarium sp. expands slowly sink injured parties and shrivel, taking shapes of concentric rings, as the fabric dries. Lesions emerges mushroom mycelium (11). Furthermore, *Penicillium*, considered a fungus postharvest produce mycotoxins that damage the fruit, flowers and seeds of plants. This fungus penetrates through lenticels or openings in the bark, which initially wound presents a white and watery consistency aspect and eventually collapses on itself (8).

A wide range of microorganisms (filamentous fungi, yeasts, bacteria, viruses and viroids) has been identified as pollutants in plant tissue culture. Contaminants can be introduced to the explant during handling in the laboratory (12) or by endophytic bacteria (13).

These endophytic and epiphytic pathogens can interfere with the development of tissue culture and compete for nutrients, whereby the microbial contamination at the base of the explant or around it, constitute a large problem *in vitro* (14) crop, so do not rule out infection by fungi isolated in this study come from inside the minitubers. Furthermore, it has been reported that these two fungal genera are frequently detected in the plant tissue culture (15).

Most pollution problems in the potato, not only *in vitro*, but also in the field, is due to vegetative propagation of plants where pathogens and diseases are transmitted from generation to generation (16), further highlighting the importance to obtain seed tubers with high quality plant.

CONCLUSIONS

Fusarium and *Penicillium* are fungal contaminants identified in the *in vitro* culture of apexes of potato in this study, resulting in the total contamination of explants, so it is important the health of plant material that tissue culture begins to the success of this system.

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Received: April 28th, 2015
Accepted: February 3rd, 2016

Revista Cultivos Tropicales
Instituto Nacional de Ciencias Agrícolas