



MICROBIOLOGICAL IMAGE

Nuclear acridine orange fluorescence in *Rhizoctonia* isolates from rice



Observación de núcleos de aislamientos de *Rhizoctonia* de arroz por fluorescencia con naranja de acridina

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Received 12 December 2014; accepted 26 February 2015

Available online 13 May 2015

The genus *Rhizoctonia* DC (1805) has long been studied as an important soilborne pathogen that causes a wide variety of symptoms because it is a non-specialized pathogen³. *Rhizoctonia sensu lato* is characterized by the lack of conidiogenous cells and this taxon is composed of two groups based on the number of nuclei per cell: the multinucleate group that belongs to *Rhizoctonia s. str.* and the binucleate group that belongs to *Ceratohiza*⁵. Currently, other authors consider the group a *Ceratobasidium–Rhizoctonia* complex⁷ and divide it into two groups: BNR (binucleate *Rhizoctonia*-like) and MNR (multinucleate *Rhizoctonia*-like)⁹. Many methods are used to observe the number of nuclei in fungal cells, e.g. safranin O, aniline blue, HCl-Giemsa. Some of these methods apply a staining solution involving laborious, time-consuming procedures that require no equipment (Fig. 1). Other methods use fluorophores, which are rapid and precise^{1,2,4,6,8,10}.

Since a reliable and rapid method was needed to explore the MNR and BNR groups associated with rice crops in Argentina, we applied an accurate technique to observe the number of nuclei in the strain cells belonging to the *Ceratobasidium–Rhizoctonia* complex.

Small portions of mycelia grown on PDA for 24h were submerged in 0.01% acridine orange (Sigma–Aldrich, USA) aqueous solution during 10s. The method applied was a modification of the Yamamoto and Uchida's staining method¹¹, the latter consisting of veronal buffer. The treated mycelium was observed under epifluorescence light using an OLYMPUS BX 51 microscope (Olympus, Japan). Digital photographs were taken using the Cool Snap-Pro System (Media Cybernetics, USA) (Fig. 2). The nuclei showed green fluorescence and the other cell components orange fluorescence. Thirty isolates from a collection of 36 were multinucleate and 6 were binucleate.

In comparison with the other methods mentioned above, the present method is characterized by easy handling and lower cost.

To our knowledge, this is the first time this methodology has been applied to observe nuclei in cells of *Rhizoctonia* isolates obtained from rice cultivars from Corrientes province, Argentina.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

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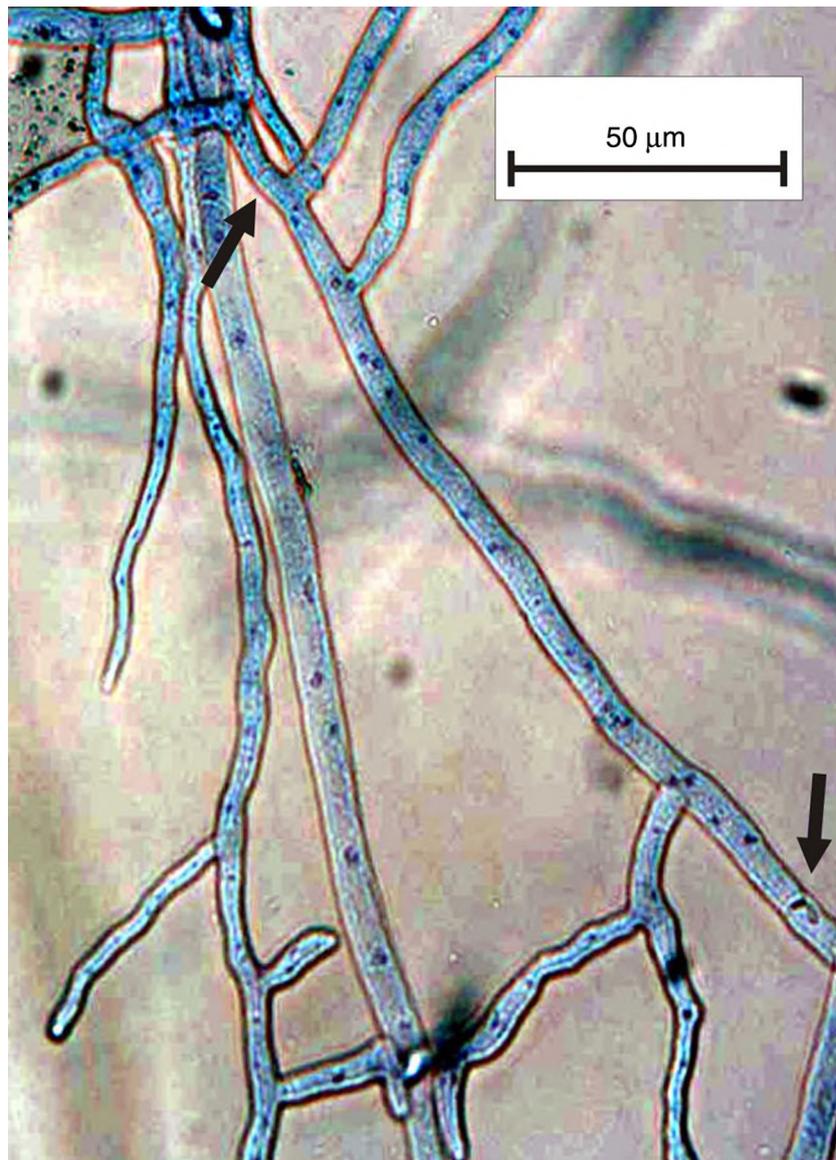


Figure 1 Multinucleate cells stained with lactophenol cotton blue. The arrows show the position of the septa.

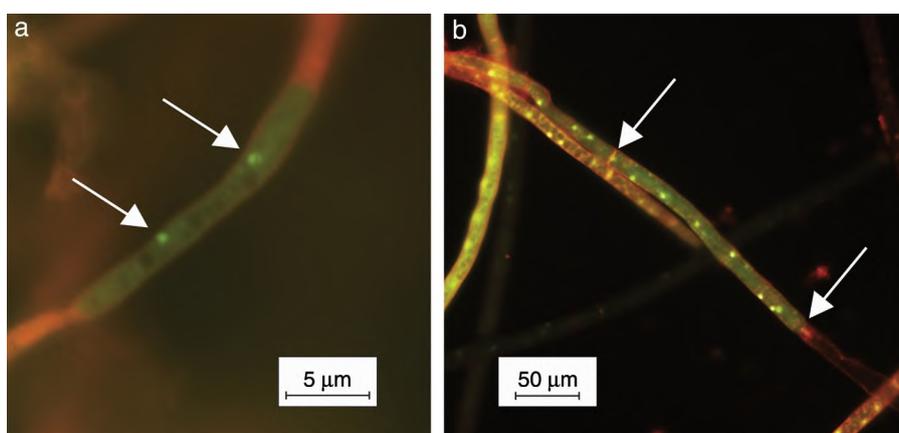


Figure 2 Cells stained with acridine orange: (a) binucleate isolate; (b) multinucleate isolate. The arrows show the nuclei.

Confidentiality of data. The authors declare that no patient data appear in this article.

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Funding, Financial support and acknowledgements

The present research was supported by the Governments of Argentina (Instituto Nacional de Tecnología Agropecuaria) and Japan (Japan International Cooperation Agency) in a bilateral collaboration program.

Conflict of interest

The authors declare that they have no conflicts of interest.

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