doi:10.20396/revpibic262018180

XXVI Congresso de Iniciação Científica Unicamp
17 a 19 de outubro Campinas | Brasil

Functional characterization of potential antifungal agents based on molecules derived from diphenylamides on Moniliphthora perniciosa, etiological agent of cacao Witches' Broom Disease

Bárbara A. Pires (IC), Mario R. O. Barsottini (PG), Gonçalo A. G. Pereira (PQ).

Abstract
Moniliophthora perniciosa is a fungal pathogen and causal agent of Witches' Broom Disease of cocoa (Theobroma cacao), which generates large losses for the cocoa culture. Currently, there is no effective means to control this disease, but the enzyme Alternative Oxidase (AOX) is a promissing target for the development of novel fungicides against M. perniciosa. Here, our aim is to develop a methodology to evaluate the antifungal activity of AOX inhibitors in vitro through growth measurements of M. perniciosa in liquid culture medium.

Key words:
Witches' Broom Disease, Diphenylamides, Enzyme Alternative Oxidase

Introduction

Fungal diseases are the main cause of losses in cocoa culture and the major threat in Brazil is the basidiomycete fungus *Moniliophthora perniciosa*, causal agent of the Witches' Broom Disease in cocoa. Studies on this disease indicate that the mitochondrial enzyme Alternative Oxidase (AOX) is an important target for the control of this pathogen (1) and our group has developed a set of AOX inhibitors with putative antifungal activity. However, we observed that growth assays in solid culture medium is not robust enough to quantitatively evaluate the biological effect of our compounds. Therefore, a new experimental procedure based on growth in liquid culture medium and measurement of M. perniciosa mycelial dry weight is being standardized. Here, we present preliminary results with the fungicide azoxystrobin (inducer of AOX).

Results and Discussion

For accurate evaluation of inhibitors activity, the dry weight of *M. perniciosa* grown in liquid culture medium was measured. For this, *M. perniciosa* was initially cultivated for 5 days in liquid medium, then the mycelium was fragmented by mechanical stirring using 0.5 cm glass beads. The mycelium was transferred to fresh culture medium, where it was maintained for 4 days for recovery. Then, 0.5 mL aliquots of the inoculum were transferred into 50 mL falcon tubes containing 5 mL of culture medium plus varying concentrations of azoxystrobin and DMSO as control. After 5 days of growth with azoxystrobin, the mycelium was washed, lyophilized and weighed. Azoxystrobin inhibited almost completely the growth of the fungus, making it impossible to evaluate its combination with AOX inhibitors. Thus, new assays with lower concentrations of azoxystrobin, based on previously executed solid media assays, were performed to find a condition in which the fungus depends on AOX, but its grown is not affected too much **(Image 1).**

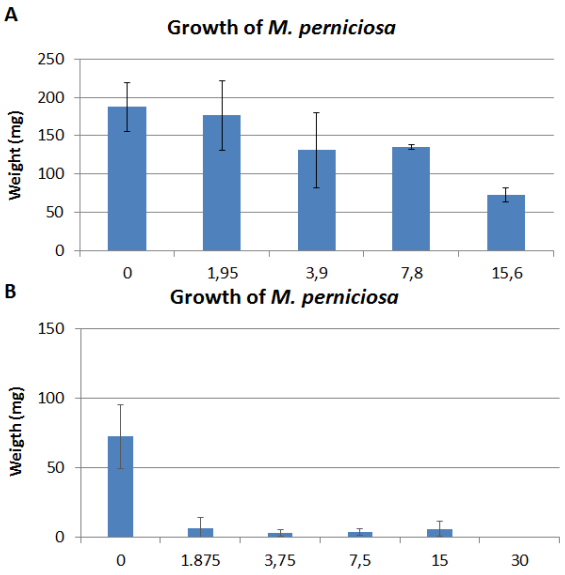

Image 1. Growth of *M. perniciosa* in the presence of azoxystrobin. In A, it is possible to observe the reduction in growth of the *M. perniciosa* in relation to the control (DMSO 0,5%), in all concentrations of azoxystrobin except in 1,95 ug/L. In B the growth is reduced from concentrations equal to 1,875 µg/L.

Conclusions

The proposed methodology should be improved since variations in the sensitivity of the *M. perniciosa* to azoxystrobin have been observed, making evaluation of the molecules activity impracticable.

Acknowledgement

This work was supported by National Counsel of Technological and Scientific Development (CNPq) and São Paulo Research Foundation (FAPESP).

Thomazella DPT, Teixeira PJPL, Oliveira HC, Saviani EE, Rincones J, Toni IM, et al. The hemibiotrophic cacao pathogen Moniliophthora perniciosa depends on a mitochondrial alternative oxidase for biotrophic development. New Phytol. 2012;194(4):1025–34.