In vitro antagonism of native Guatemalan isolates of *Trichoderma harzianum* and *T. viride* for biological control of *Rhizoctonia solani*

José A. Ruiz-Chután[1, 2], Julio E. Berdúo-Sandoval [1], Marie Kalousová [2], Bohdan Lojka [2], Amílcar Sánchez-Pérez [1]

1 Dirección General de Investigación, Universidad de San Carlos de Guatemala

2 Czech University of Life Sciences Prague, Faculty of Tropical AgriSciences

Introduction

Rhizoctonia solani is a basidiomycete present in the soil[1] that currently affects the production of potato crops in Guatemala's western highlands, causing the disease known as black scurf. The current classification of *R. solani* is based on the ability of the hyphae to merge and form an anastomosis group (AG), 13 AG being recognized so far [2]. Due to the negative impacts of chemical disease control, a viable alternative is presented in usage of fungi from the genus *Trichoderma* as a biological control strategy [3]. The research objective was to identify the AG of *R. solani* present in the western highlands of Guatemala and to evaluate the potencial of native isolates of *T. harzianum* and *T. viride* as biological control agents.

Methodology

From three potato-producing departments (Figure 1), we obtained 78 *R. solani* isolates. DNA was extracted from the isolates and specific PCR was conducted to identify the AG. 25 and 29 isolates of *T. harzianum* and *T. viride*, respectively were fingerprinted with AFLP. Data analysis: Percentage Inhibition of Radial Growth (PIRG)- Student's t-test, ANOVA and post hoc analysis (ggstatsplot[4]), radial cluster dendrogram (ape [5]).

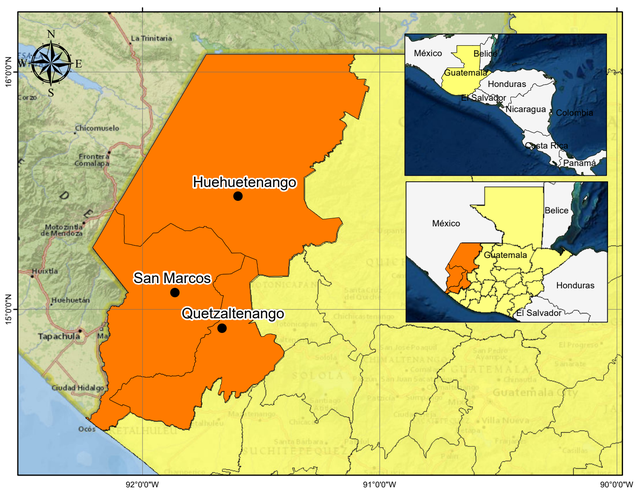

Figure 1. Collecting sites

Results

Of the 78 isolates of *R. solani*, the groups AG-3PT, AG-4-HG-II and AG-5 were identified (Fig 2a) with an incidence of 46.16%, 14.10% and 17.96%, respectively, while 17 isolates could not be assigned (Fig 2b).

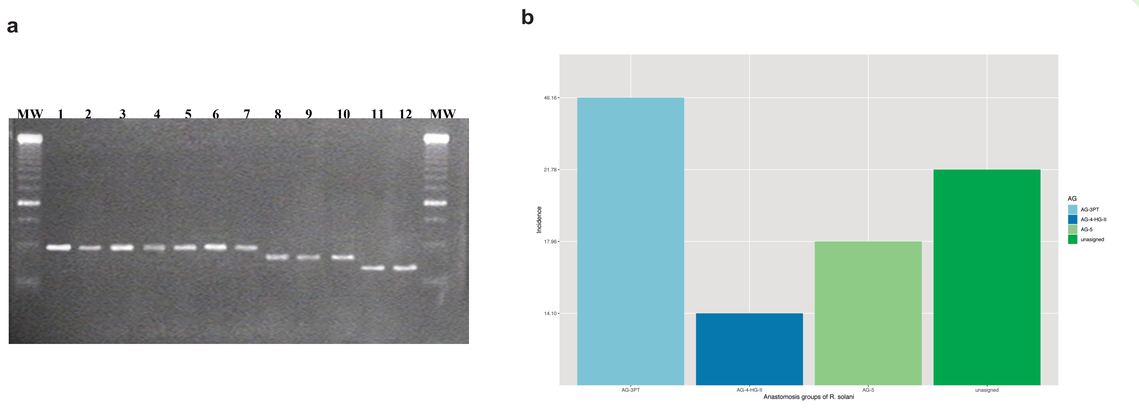

Figure 2. a) Agarose gel electrophoresis of PCR-amplified products from the isolates of *R. solani*. Lane MW= 250 bp DNA ladder. Lanes 1-7 AG-3PT positive (480 bp). Lanes 8-10 AG4-HGII positive (420 bp). Lanes 11 and 12 AG-5 positive (350 bp) . b) Incidence of the anastomosis groups of *R. solani*.

Acknowledgments

The study was supported by Dirección General de Investigación (Digi) through project No. B18-2020

Isolates of *T. harzianum* showed a higher average Percentage Inhibition of Radial Growth (PIRG) of *R. solani* (56.71 %) than *T. viride* (37.58 %) ($p < .001$) (Fig 3).

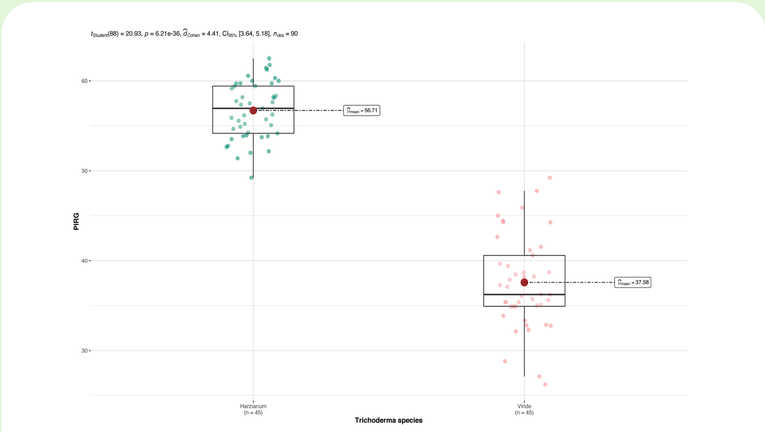

Figure 3. PIRG comparison between *T. harzianum* and *T. viride*

We determined that the genetic groups of *T. harzianum* and *T. virens* (Fig 4a) are equally effective against the 3 AG identified (Fig 4b, 4c), highlighting the benefit of using native isolates.

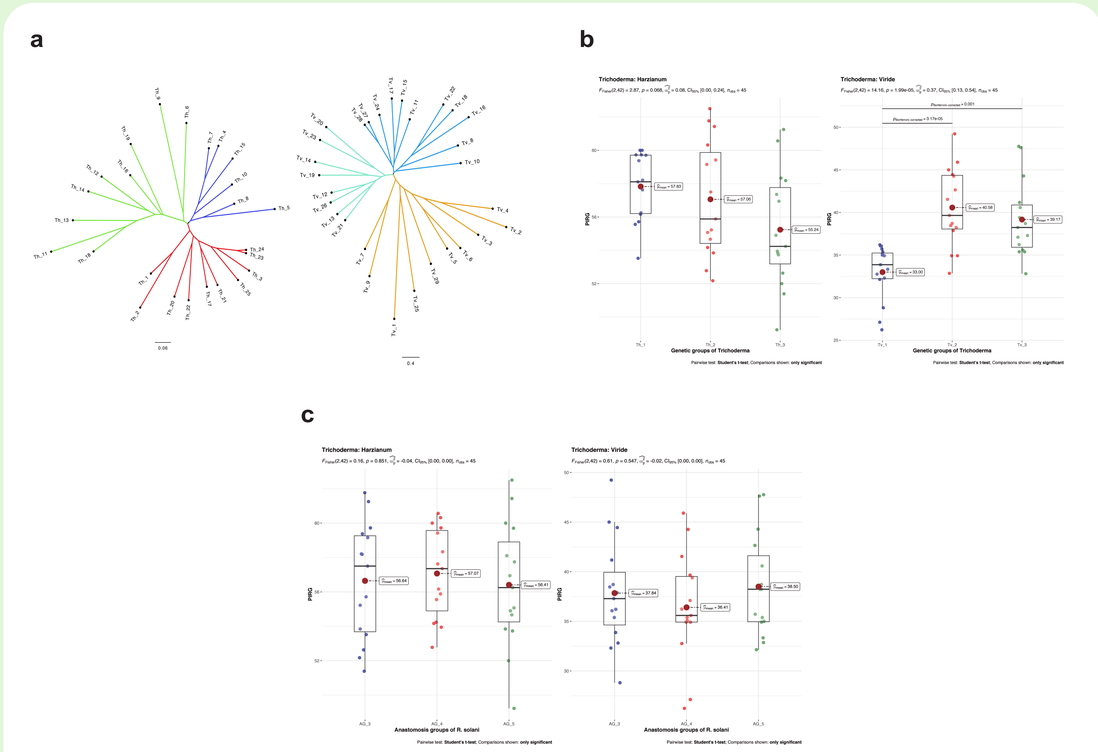

Figure 4. *a)* Radial cluster dendrogram showing the genetic relationship of the 25 and 29 isolates of *T. harzianum* and *T. viride*, respectively. Branch color depicts different genetic groups. *b)* PIRG by each genetic group of both *Trichoderma species*. *c)* PIRG caused by *T. harzianum* and *T. viride* over each anastomosis group.

Conclusions

We demonstrate the in vitro potential of native isolates of *T.harzianum* and *T. viride* to inhibit the growth of the 3 AG identified. We suggest using sequencing of the ITS region for the identification of unassigned isolates of *R. solani* through sequence comparison of NCBI genebank sequences and field evaluations to assess the efficiency of *T. harzianum* and *T. viride* native isolates.

References

[1] Rajendraprasad, M., Vidyasagar, B., Uma, G., & Koteswar, R. (2017). In vitro evaluation of fungicides and biocontrol agents against *Rhizoctonia solani* in tomato. International Journal of Plant & Soil Science, 17(5), 1–9.

[2] Carling, D. E., Kuninaga, S., & Brainard, K. A. (2002). Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among sunsets of *Rhizoctonia solani* anastomosis group 2 and AG BI. Phytopathology, 92, 43–50.

[3] Abbas, A., Jiang, D., & Fu, Y. (2017). *Trichoderma spp* . as antagonist of *Rhizoctonia solani*. Journal of Plant Pathology & Microbiology, 8(3), 1–9.

[4] Patil, I. (2018). ggstatsplot: 'ggplot2' Based Plots with Statistical Details. CRAN. Retrieved from: https://cran.r-project.org/web/packages/ggstatsplot/index.html

[5] Paradis, E., Schliep K. (2019).ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics,(35),526–528.