How viruses affect their host, the panglobal pathogen *Phytophthora cinnamomi* in vitro?

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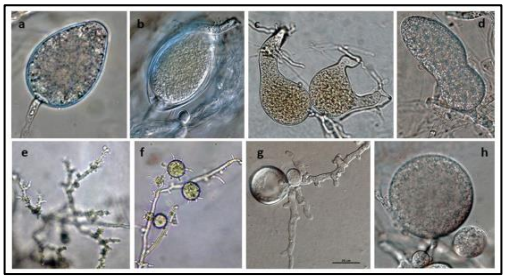
Introduction

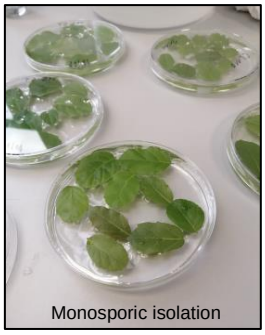
Members of genus *Phytophthora* are oomycetes belonging to the kingdom Stramenipila (Beakes et al., 2012; Thines and Choi, 2016). Most known members are primary plant and forest pathogens causing important economic losses in agriculture, horticulture and the forestry industry, threatening natural ecosystems and biodiversity on a global scale (Kroon et al., 2012). Arguably the most notorious species that belongs to genus *Phytophthora* is a soil-borne pathogen *P. cinnamomi*, which reached a worldwide distribution (Kinal et al., 1993). It infects close to 5000 species such as rhododendron, oak, chestnut, pine, avocados, etc. (Moreira and Martins, 2005). *Phytophthora spp*., and in particular, *P. cinnamomi*, harbour multiple viral infections and certain type of viruses seem to be very bound to their hosts (Botella et al. 2020; Botella and Jung, 2021). Since many mycoviruses are capable to cause hypovirulence, a reduction of virulence, in pathogenic fungi and oomycetes, these viruses can be potentially used as biocontrol agents (BCAs) (Milgroom and Cortesi, 2004).



Phytophthora cinnamomi selected specimen P6490 asexual phase (a-h): (a-d) different shapes of persistent sporangia, (e) typical coralloid mycelia, (f, g) hyphal swellings, (h) chlamydospore and hyphal swellings; photos by Gloria Abad, USDA-APHIS-PPQ.

Methodology

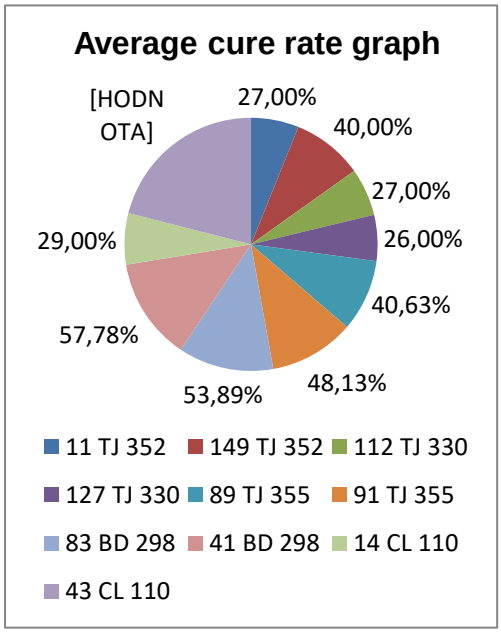
The samples used for this project were already confirmed to host various viruses by Dr. Leticia Botella's team. To obtain isogenic virus-free isolates, which was the aim of this project, different methods were used, such as monosporic isolation or the use of chemicals (PEG, Ribavirin, Cycloheximidine). Monosporic isolation also allows us to estimate the vertical transmission rates of the viruses hosted by the original isolate. RNA extraction and RT-PCR using specific primers were then carried out to confirm the presence of the viruses in each monosporic culture.



Monosporic isolation

Results

The first monosporic isolation was not 100% successful, so selected samples were treated with PEG. However, this method proved to be ineffective, and therefore the selected isolates were re-treated using the monosporic isolation method. The graph of the average cure rate shows how successful this method of virus elimination was in different samples. The highest success rate, achieved in sample 43 CL 110, was 93%, while the lowest success rate, achieved in sample 127 TJ 330, was only 27%. Also, Phytophthora cinnamomi ormycovirus 7 had the highest vertical transmission rate and was eliminated at 83.13%, while Phytophthora cinnamomi ormycovirus 3 had the lowest vertical transmission rate and was eliminated at only 9.38%.



Conclusion

Of the methods of virus elimination used so far, monosporic isolation has proven to be more effective than the use of PEG according to the experiments performed. Monosporic isolation also proved to be more effective upon repetition. Phytophthora cinnamomi ormycovirus 3 proved to be the most abundant virus and was the most difficult to eliminate. On the other hand, the least abundant virus was Phytophthora cinnamomi ormycovirus 7, which was eliminated at 83.13%.

Acknowledgement

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