

VIRUS ELIMINATION METHODS IN *PHYTOPHTHORA CASTANEA*

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SUMMARY

Knowledge of fungal and oomycete virology is expanding, and numerous studies have been conducted not only to discover novel species but also to improve our understanding of the biological interaction between viruses and their fungal and oomycete hosts. In the oomycete genus *Phytophthora*, virus research is still an emerging field, and very few viruses have so far been described. Likewise, the role of viruses in this plant pathogenic genus is poorly understood. To determine the effects of viral species on their host pathogens, the crucial step is designing a convenient method for obtaining virus-free isolates. This research aims to develop efficient methods that eliminate viruses in virus-hosting isolates of *Phytophthora castaneae* thus enabling the further study of virus-host interactions. These methods include the usage of the antiviral drug ribavirin combined with hyphal tip culturing and matrix induced water stress by polyethylene glycol (PEG). According to the literature, usage of polyethylene glycol (PEG)-induced matrix potential gives good results in eliminating viruses in fungi (Thapa et al., 2016). Ribavirin is used for elimination of viruses in fungal (Thapa et al., 2016) but also in oomycete plant pathogenic species (Cai et al., 2019). However, the successfulness of any treatment is species-specific and virus-specific. We aimed to find the most efficient method and the most efficient concentration of the above-mentioned substances that would cure isolates that are infected by multiple viruses from different viral genera but as well isolates infected by single virus species.



Water Potential	PEG g/g H ₂ O (20°C)	g PEG/ 50ml	g PEG/ 300ml
-5 Mpa	0.1925	9.625	28.875
-6 Mpa	0.21237	10.6185	63.711
-7 Mpa	0.2307	11.535	69.21
-8 Mpa	0.24774	12.387	74.322
-9 Mpa	0.26376	13.188	79.128
-11 Mpa	0.29333	14.6665	87.999
-13 Mpa	0.32	16	96
-15 Mpa	0.345	17.25	103.5
-20 Mpa	0.4013	20.065	120.39
0 Mpa	0	0	0



MATERIALS AND METHODS

- (i) Chemotherapy combined with single tip isolation
- (ii) PEG induced matrix stress on water availability
- (iii) PEG induced matrix stress on water availability combined with chemotherapy and single hyphal tip isolation



RESULTS

The isolates obtained after these treatments were grown on membrane supplied agar plates to produce a sufficient amount of mycelia for total RNA extraction. The extracted total RNA was converted to complementary DNA by reverse transcriptase and then the cDNA is used as a template in PCR reaction. The presence or absence of viruses will be checked by virus-specific primers.

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