Infectivity decline of an RNA plant virus by increased mutagenesis supports the lethal defection model *in vivo*

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Lethal mutagenesis is a new antiviral therapy based on increasing the mutation rate by using mutagenic base and nucleoside analogues whose molecular mechanisms are not fully understood. Most of the research has been conducted on animal RNA viruses in cell culture and, to a lesser extent, *in vivo*. There is experimental evidence supporting the model of lethal defection for lethal mutagenesis of RNA viruses. In this model, viral genomes with a low degree of mutation and low specific infectivity, termed "defectors", exert an interfering activity leading to virus loss. Lethal mutagenesis of plant viruses has not been addressed yet despite being excellent in vivo model systems that develop systemic infections, undergo rapid bottlenecks and pose no ethical issues.

Here, we address lethal mutagenesis *in vivo* of Tobacco mosaic virus (TMV), a single-stranded positive RNA virus of 6.4 Kb. *Nicotiana tabacum* plants cultured *in vitro* were treated with 25, 50 and 100 µg/ml of the base analogue 5-fluorouracil (FU) and 24 h later were inoculated with 50 lesion forming units (lfu) of TMV. We analyzed the infectivity, viral load and mutant spectra of viral populations after 5 and 10 days of treatment, as well as of populations that went 10 days of treatment followed by 21 days of *ex vitro* growth in the absence of FU.

The results show that TMV infectivity decreases when treated with 50 and 100 µg/ml FU for 10 days. TMV mutagenized populations grown without FU reach infectivity values higher than untreated populations. Predominant mutations in FU-treated populations with decreased infectivity at 10 dpi are U→C, A→G and G→A transitions, which are expected due to the action of FU. TMV replication is not affected by FU at any dose and there are no imbalances of ribonucleotide triphosphate pools measured by HPLC. No differences in mutation frequencies and Shannon Entropies between control and FU-treated populations with declined infectivity were found. However, we did found a dose-dependent decrease of specific infectivity in FU-treated populations, but not in untreated samples, as well as dominance of molecules with a low degree of mutation. Specific infectivity recovered to control levels after 21 days of growth without the analogue. Altogether, our results suggest that TMV defector molecules mediate the decrease in TMV infectivity. This is the first report that addresses the molecular basis of lethal defection *in vivo* using an RNA plant virus.