Infectivity decline of an RNA plant virus by increased mutagenesis *in vivo*

Luis Díaz-Martínez, Isabel Brichette Mieg and Ana Grande-Pérez

Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” (IHSM-UMA-CSIC), Área de Genética, Universidad de Málaga, Campus de Teatinos, 29071 Málaga, Spain.

According to the lethal defection model, during lethal mutagenesis viral genomes with a low degree of mutation and low specific infectivity exert an interfering activity that leads to virus loss. Lethal mutagenesis of plant viruses has not been reported to date. We address lethal defection *in vivo* of Tobacco mosaic virus (TMV), a single-stranded positive RNA virus. *Nicotiana tabacum* plants cultured *in vitro* were treated with 25, 50 and 100 µg/ml of the base analogue 5-fluorouracil (FU) and 24h later were inoculated with 50 lesion forming units of TMV.

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 transitions which are expected due to the action of FU. Viral load 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 were found. However, we 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.