A novel cloning strategy for isolating, genotyping and phenotyping genetic variants of geminiviruses

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Viruses of the genus *Begomovirus* (*Geminiviridae*) are emerging economically important plant viruses with a circular, single-stranded DNA genome. Previous studies have shown that geminiviruses and RNA viruses exhibit similar mutation frequencies, although geminiviruses are replicated by host DNA polymerases and RNA viruses by their own virus-encoded error-prone RNA-dependent RNA-polymerase. However, the phenotypic effects of naturally occurring mutations have never been extensively investigated in geminiviruses, particularly because, to be infectious, cloned viral genomes usually require sub-cloning as complete or partial tandem repeats into a binary vector from *Agrobacterium tumefaciens*.

Using *Tomato yellow leaf curl virus* (TYLCV), we show here that infectivity can be obtained when only a 41-nucleotide region containing a highly conserved stem-loop is repeated. A binary vector containing this 41-nt region and a unique restriction site was created, allowing direct cloning of infectious monomeric viral genomes provided that they harbour the same restriction site at the corresponding nucleotide position. This experimental system, which can be transferable to other geminiviruses, was validated by analysis of the phenotypic effect of mutations appearing in TYLCV genomes in a single tomato host plant originally inoculated with a unique viral sequence. Fourteen full-length infectious genomes extracted from this plant were directly cloned and sequenced. The mutation frequency was 1.38×10^{-4} mutation per nucleotide sequenced, similar to that found previously for another begomovirus by sequencing PCR-amplified partial sequences. Interestingly, even in this minimal pool of analysed genomes, mutants with altered properties were readily identified, one of them being fitter and reducing plant biomass more drastically than the parental clone.

The cloning strategy presented here is useful for any extensive phenotyping of geminivirus variants and particularly of artificially generated mutants or recombinants.