A sensitive method to quantify replicative forms of circular DNA viruses

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Although real-time PCR diagnostic protocols for detection of geminivirusesare available, none of them are able to discriminate between the two strands generated during an infection: the viral strand (VS) that is encapsidated within virions; and the complementary-sense strand (CS) that is used as template to generate more viral strands. Here we describe a two-step real-time PCR protocol to quantify the amount of VS and CS as well as how many of those strands are arranged as single or double strand (dsDNA). The method was set and tested on synthesized VS and CS circular molecules of the begomoviruses*Tomato yellow leaf curl Sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV), both involved in Tomato yellow leaf curl disease. Then the amount of VS and CS was determined in systemic infections of TYLCSV and TYLCV in tomato and *Nicotianabenthamiana* plants. The results show that the ratio VS/CS is not constant throughout the time of infection and depends on the combination virus-host. In tomato, the increment of ssDNA measured at 7 and 42 dpi in both viruses was due mostly to synthesis of VS. In both viruses more than 98% of their CS is arranged as dsDNA, while VS is disposed both as ssDNA and dsDNA. We also measured the amount of ssDNA of both polarities in *N. benthamiana* leaves agroinfiltrated with TYLCSV C2, C3, C4 and V2 mutants. The results show that C2, C4 and V2 mutants accumulate similar amounts of DNA, both as ssDNA and dsDNA, and have VS/CS ratios comparable to the wild type TYLCSV. However, the C3 mutant presents reduced amounts of all species of viral DNA. The protocol described here is a significant improvement of the techniques in use to quantify circular ssDNA and can help to understand in detail the molecular scenario during replication of any viruses whose genome is made of circular DNA.