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Biogenesis of siRNA and miRNA upon infection of *Nicotiana benthamiana* plants with a virus and its mutants

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As a result of morphogenesis features and evolutionary development pathway, among eukaryotic organisms, epigenetic variability is most effectively employed by plants. Double stranded RNA directed gene silencing (also referred as RNA-interference) is a biological mechanism present in all eukaryotes and known as one of the central anti-viral defence mechanisms in plants. In this system produced by viral RdRp double stranded RNA molecules are recognized by PTGS system and cleaved into small interfering RNA by RNase like III enzyme DICER. Following then, these siRNA molecules are loaded into RISC complex, which leads to the post-transcriptional gene degradation or "silencing arrest". Viral protein p19 encoded by Tombusviridae family member *Tomato bushy stunt virus* suppresses silencing system by binding with short interfering RNA. This protein widely used in RNA-interference studies, because it binds with high affinity to 21–24 nt dsRNA in sequence-independent manner. We used mechanic inoculation of model plants *Nicotiana benthamiana* by *Tomato bushy stunt virus* and its p19 deficient mutant to study the levels of siRNA circulation during infection. After infection of plants we isolated total RNA from tissues and polyacrylamide gel electrophoresis with urea was done to detect and segregate siRNA. Then we transferred RNA molecules to the membrane and incubated them with DIG. The purpose of this work is to study the effect of viral infection on the generation of a pool of siRNA molecules in plant tissues compared to uninfected plants.
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