ANALYSIS OF THE BEAN YELLOW DWARF VIRUS HOST INTERACTIONS

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ABSTRACT

Geminiviruses have small single-stranded DNA genomes that encode few proteins. Their replication, transcription and translation rely mostly on plant proteins. However they are able to replicate in differentiated cells where the DNA replication machinery is no longer available. Indeed, in plant cells, many regulatory mechanisms are present to prevent a second round of replication of the DNA, so that the differentiated cells stay in G1 phase. How geminiviruses are able to infect such cells and to switch on the cell cycle machinery again is a question of fundamental interest. Some geminiviruses induce the expression of proteins necessary for G1 to S phase transition although they do not allow the progression to continue through G2 to mitosis. However, other geminivirus infections result in additional rounds of cell division.

The aim of this project is to use *Bean yellow dwarf virus* (BeYDV) a member of the *Mastrevirus* genus of the family *Geminiviridae* in order to identify host gene targets of the virus Rep and RepA proteins involved in replication and transcription. This virus has been chosen for its ability to infect *Arabidopsis thaliana.*

Rep and RepA are likely to be toxic *in planta,* which is why the estrogen inducible expression system was used for transformation of *Arabidopsis*. Plants transgenic for *Rep*, *RepA*, and for *GUS* were obtained (designed XVE-*Rep*, XVE-*RepA*, and XVE-*GUS*, respectively). The expression pattern obtained from the estrogen inducible promoter has been assessed using one XVE-*GUS* line. Three lines (XVE-*RepA*10, XVE-*Rep*3 and XVE-*Rep*15) show a stress-response-like phenotype when induced.
The phenotypes of these induced lines have been correlated with the expression of the transgenes *Rep* and *RepA*. Using Northern Blot and reverse transcription followed by PCR (RT-PCR) techniques, it was demonstrated that the transgenes *Rep* and *RepA* are only expressed after induction. The lines XVE-*Rep*3 and XVE-*RepA*10 were chosen for further analyses.

Changes in host gene expression mediated by *Rep* and *RepA* have been investigated by RT-PCR and real time PCR. The results from the real time PCR analysis showed that the candidate gene *PCNA At2* might be up-regulated after 4 to 6 hours of *RepA* induction but was not affected by the *Rep* expression. Crosses of the transgenic lines with cell cycle GUS reporter gene showed that *RepA* is affecting the expression pattern in the cotyledons of induced plants. Flow cytometry analyses on the induced transgenic lines suggested that *RepA* expression might induce endoreduplication in the cotyledons.

When Rep and RepA were fused to the Green Florescent Protein (GFP) only Rep localised into the nucleus. *In situ* hybridisation showed that BeYDV was vascular-restricted in *Nicotiana (N.) benthamiana*, and SEM analysis showed that the size of epidermal cells of *N. benthamiana* leaves was affected by BeYDV infection.