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► **To cite this version:**

Diane Bortolamiol-Bécet, Véronique Brault, Véronique Ziegler-Graff. Phloem specific Virus-Induced Gene-Silencing using a Recombinant polerovirus. 13ième Rencontre de Virologie Végétale, Jan 2011, AUSSOIS, France. , pp.13ième Rencontre de Virologie Végétale, 2011. hal-02808004

HAL Id: hal-02808004

<https://hal.inrae.fr/hal-02808004>

Submitted on 6 Jun 2020

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8- Phloem specific Virus-Induced Gene-Silencing using a Recombinant polerovirus

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Virus Induced Gene Silencing has proven to be a powerful tool for the study of gene function in plants. We have taken advantage of polerovirus phloem restriction to engineer a chimeric virus carrying a cDNA fragment from an endogenous plant gene to specifically silence the gene in vascular tissues. Sense (S), antisense (AS) and inverted-repeat (IR) sequences of a portion of the *AtCHLII* gene required for chlorophyll biosynthesis were inserted into the 3' non-coding region of *Turnip yellows Virus* (TuYV) and the corresponding viruses were agroinoculated to *Arabidopsis thaliana*. A vein chlorosis phenotype was observed as early as nine days post-inoculation for all chimeric viruses, and further intensified with plant growth during the following weeks while remaining restricted to the vasculature. When similar constructs were introduced into virus carrying a knock-out mutation in the silencing suppressor P0, a similar phenotype was observed following agroinoculation, although there was a short delay compared to WT derived viruses. Thus P0 has no major impact on generation, propagation and action of short distance silencing signals.

Interestingly, a more durable phenotype was recorded with the S and AS constructs than with the IR containing viruses. Molecular analysis of the progeny virus revealed lower stability of the insert in the IR recombinant viruses. Experiments of aphid transmissibility were performed with either S or IR recombinant TuYV and confirmed these data: extensive clearly visible vein chlorosis was observed on plants infected with the S chimeric TuYV while plants infected with the IR recombinant virus showed only faint and sporadic spots of chlorosis along the veins. Molecular analysis further revealed that the S recombinant TuYV genome was stable even after aphid transmission. This study shows that a recombinant polerovirus TuYV containing a host gene sequence can be used to efficiently silence homologous genes specifically in phloem tissue. It constitutes the first example of a labelled and systemically infectious polerovirus where the spatial and temporal progression of the viral infection can be visually monitored.