Measuring viral load by fluorescence imaging: validation of a nondestructive method to phenotype plant resistance to virus accumulation

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Breeding for plant resistance to viruses with conventional approaches remains a long and expensive process. In addition, it requires reliable methods to measure the efficiency of virus limitation in resistant genotypes. Such limitation may be assessed via the viral load at a given point of time, which can easily be measured using serological (e.g. quantitative ELISA) or molecular (e.g. RT-qPCR) techniques. However, because these methods are destructive, monitoring the temporal dynamics of viral accumulation in infected leaves remains a challenge.

To tackle this issue, we assessed the reliability of fluorescence imaging, a non-destructive method, for approximating virus accumulation in plants. To this end, we first carried out an experiment based on the pathosystem pepper-potato virus Y (PVY, *Potyvirus*) using a modified virus that expresses a Green Fluorescent Protein (GFP) to compare 2 quantification methods: semi-quantitative ELISA and fluorescence imaging. Secondly, we evaluated the protocol based on fluorescence imaging to monitor the kinetics of virus accumulation in 'Yolo Wonder' and 'Perennial', two well-known genotypes of pepper (*Capsicum annuum*) showing contrasted levels of resistance to PVY.

The major result indicated a significant correlation between the proportion of the leaf surface emitting fluorescence (linked to the expression of the GFP protein in infected cells) and the concentration of virus coat protein measured by quantitative ELISA. The protocol based on fluorescence imaging could thus be used to monitor viral accumulation in two pepper cultivars and highlighted contrasted kinetics. This approach will be applied in further experiments to phenotype pepper resistance to PVY.