Rapid and Quantitative Molecular Assays for Investigating Virus-Host and Virus-Virus Interactions using Negative-Stranded RNA Viruses as a Model System

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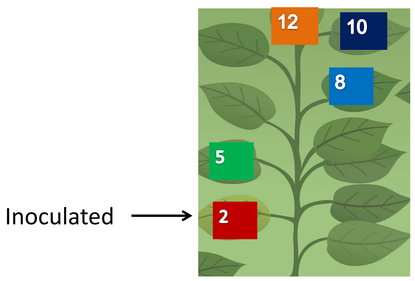
Introduction

- The genus *Tospovirus* (*Bunyaviridae* family) has some of the most economically important viruses that cause significant losses in a wide range of vegetables and ornamental crops worldwide.
- *Tomato spotted wilt virus* (TSWV) and *Iris yellow spot virus* (IYSV) are among the most damaging viruses in this genus
- Tospoviruses have a tripartite, single-stranded RNA with negative polarity.
- Rapid and sensitive detection of these viruses is an important tactic toward disease management.
- Real-time PCR is one of the most effective methods currently being used for plant virus diagnostics
- In this research , a real-time PCR based assay was developed to investigate the distribution of IYSV and TSWV when they are either individually, or co-inoculated, in the model differential host *Datura stramonium*. This assay was also used to determine virus-host and virus-virus interactions.

Materials and Methods

- *D. stramonium* plants were inoculated with IYSV, TSWV, or co-inoculated with TSWV and IYSV at 30 days after sowing.
- Samples were collected from both inoculated (leaf 2) and non-inoculated leaves (5, 8, 10 and 12) every 7 days, for 35 days.
- Total RNA isolation was done, followed by cDNA synthesis
- Real-time PCR standard curves for each virus were created.
- The concentration of each experimental sample was determined in real-time PCR based on the established standard curves.

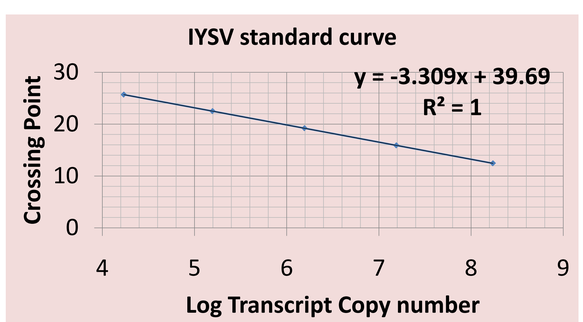


Position and color-coding for leaves sampled

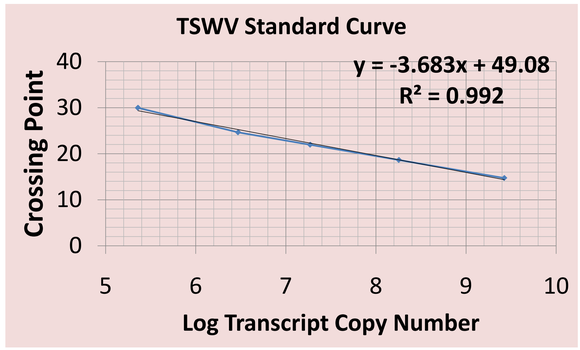
Results

Real –time PCR Standard Curves

- Real-time PCR standard curves were established for both IYSV and TSWV with 100% and 98.2% amplification efficiency, respectively.

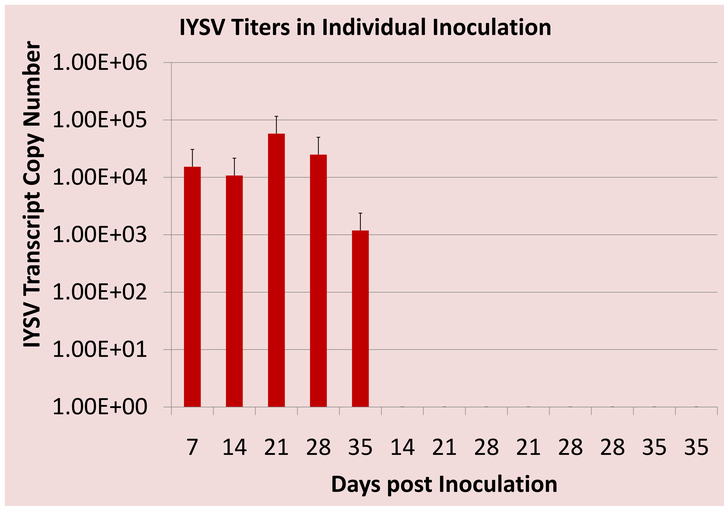


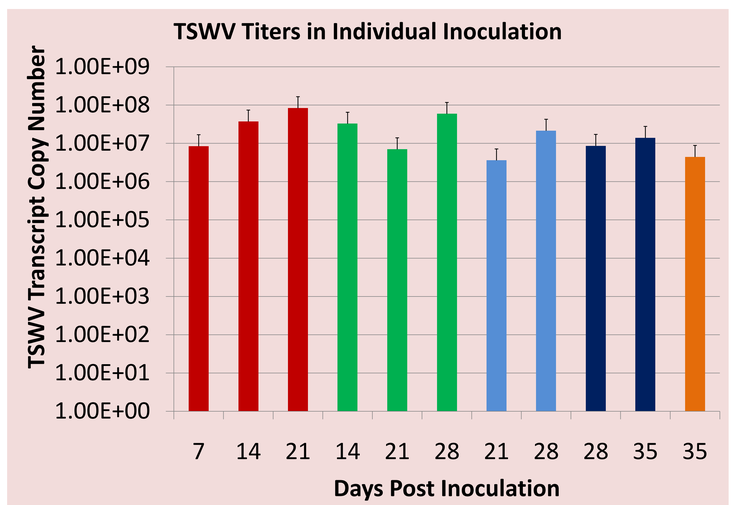
Results



IYSV and TSWV Assays

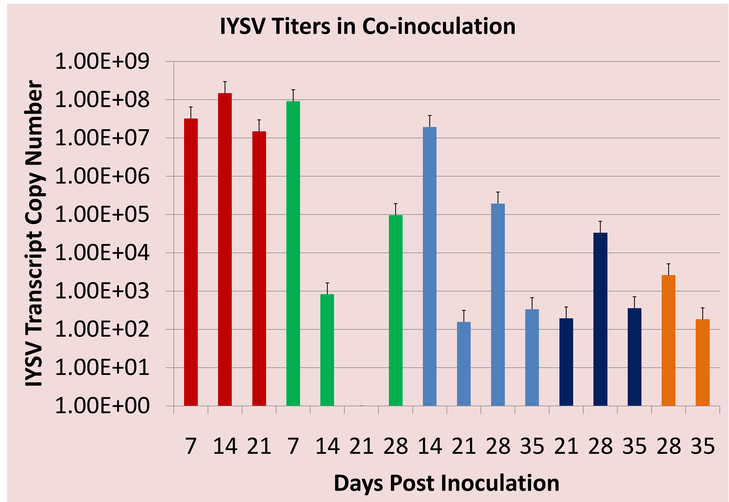
- Real-time PCR confirmed that IYSV infection remains localized to inoculation point.
- TSWV replicates and spreads to all non-inoculated leaves
- On average, TSWV titers are higher than IYSV titers.





Co-inoculation Assays

- TSWV was still detected in all the leaves assayed
- Interestingly, IYSV was also occasionally detected in non-inoculated leaves in lower titers.
- Average IYSV titers increased in co-inoculated leaves.
- More severe symptoms were observed in host.



Conclusions

- TSWV complements genetic function of IYSV.
- This real-time PCR assay can be used to determine virus-virus and virus-host interactions in individual or mixed natural infections.

Acknowledgements

- I thank Fulbright for graduate assistantship.

References

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- Dietzgen et al. 2005. Annals of Applied Biology 146: 517 – 530.
- Boonham et al. 2002. Journal of Virological Methods 101: 37 -48

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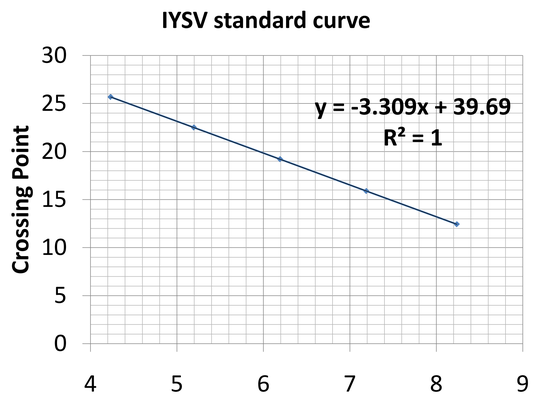
IYSV standard curve
y = -3.309x + 39.69
R² = 1
Crossing Point
30
25
20
15
10
5
0
4
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6
7
8
9
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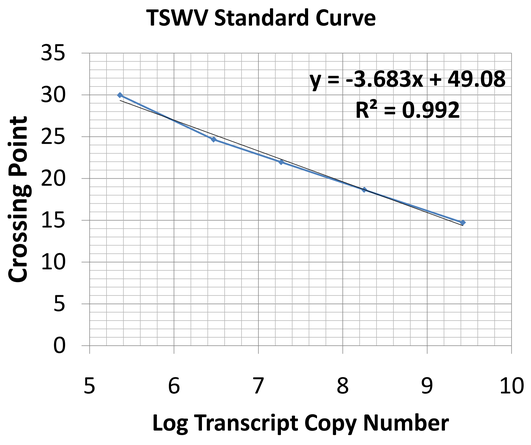
TSWV Standard Curve
y = -3.683x + 49.08
R² = 0.992
Crossing Point
35
30
25
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6
7
8
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