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.

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.

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.

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.

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References

IYSV standard curve

\[ y = -3.309x + 39.69 \]

\[ R^2 = 1 \]

TSWV Standard Curve

\[ y = -3.683x + 49.08 \]

\[ R^2 = 0.992 \]