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Induction and suppression of antiviral RNA silencing by Tomato spotted wilt virus

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Induction and suppression of antiviral RNA silencing by *Tomato spotted wilt virus*

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INTRODUCTION

RNA silencing is an essential antiviral defense system in plants. Triggered by double-stranded RNA, silencing results in degradation or translational repression of target RNA. Viruses are inducers and targets of RNA silencing. To condition susceptibility, most plant viruses encode silencing suppressor proteins that interfere with RNA silencing. *Tomato spotted wilt virus* (TSWV) NSs protein is an RNA silencing suppressor. The mechanism of RNA silencing suppression by NSs and its role in virus infection and movement remain to be determined. We cloned NSs from the Hawaii isolate of TSWV. Using two independent assays, we show that NSs restored pathogenicity and supported the formation of local infection foci by suppressor-deficient *Turnip mosaic virus* (TuMV) and *Turnip crinkle virus* (TCV). Suppression of silencing directed against heterologous viruses establishes the foundation to determine the mechanism of antiviral RNA silencing suppression by NSs.

METHODS

VIRUS. TSWV was transmitted by thrips (*F. occidentalis*) to *Emilia fosbergii* and used to prepare inoculum. *Datura stramonium*, *Nicotiana benthamiana*, *Solanum lycopersicum* and *Arabidopsis thaliana* were mechanically inoculated (Ocampo et al., 2016)

CLONING. Virion RNA from *N. benthamiana* was used to clone two versions of NSs, one harboring and one lacking the 5' UTR, which were inserted into pENTR. One pENTR-NSs clone and one pENTR-5'UTR-NSs clone were transferred to pMDC32. By electroporation, *Agrobacterium tumefaciens* strain GV3101 was transformed with plasmids carrying NSs (pMDC32-5'UTR-NSs and pMDC32-NSs), tombusviral P19 (pCB302-P19), beta-glucuronidase (pMDC32-GUS) (Johansen and Carrington, 2001; Garcia-Ruiz et al., 2010), or the single stranded GFP (ssGFP) reporter (pPZP-35S-GFP) (Tatineni et al., 2012) and used for agroinfiltration of *N. benthamiana* leaves.

SILENCING SUPPRESION ASSAY. Suppressor-deficient TCV-GFP (Powers et al., 2008) and TuMV-AS9-GFP (Garcia-Ruiz et al., 2010) were used to measure antiviral RNA silencing by two complementary approaches: co-infiltration or infiltration followed by mechanical inoculation (Powers et al., 2008). TCV-GFP lacks the coat protein, which is a silencing suppressor (Powers et al., 2008; Qu et al., 2008), and TuMV-AS9-GFP harbors an inactivating mutation in silencing suppressor HC-Pro (Garcia-Ruiz et al., 2010). Per treatment, six plants were infiltrated. Experiments were repeated twice. In co-infiltration assays, infiltrated leaves were collected for protein and RNA extraction. In a complementary assay, suppression of antiviral RNA silencing was scored by counting the number of local infection foci per leaf.

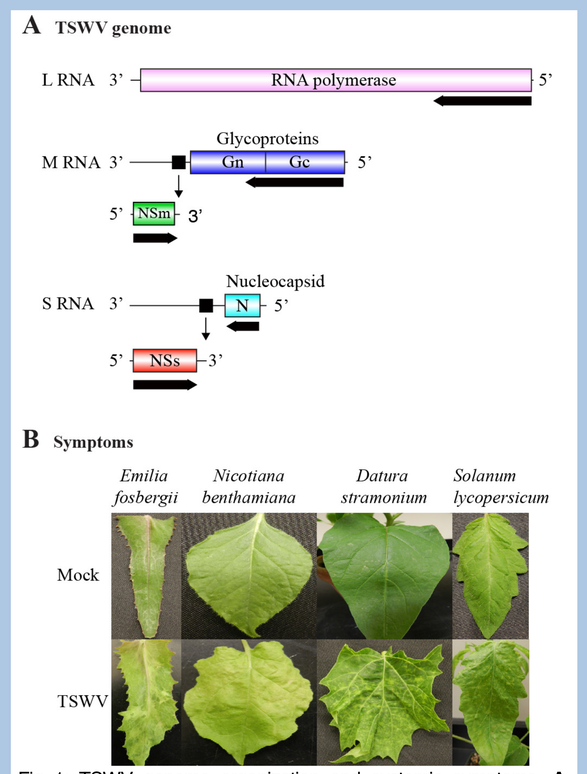

Fig 1: TSWV genome organization and systemic symptoms. **A.** Schematic representation of the TSWV genome. Colored cylinders indicate open reading frames. Lines indicate 3' and 5' UTR, and intergenic regions. Black squares indicate transcription termination hairpins. Black arrows indicate the direction of translation. Subgenomic RNAs are transcribed from M and S RNAs. **B.** Representative TSWV-induced systemic symptoms in selected hosts at 21 dpi.

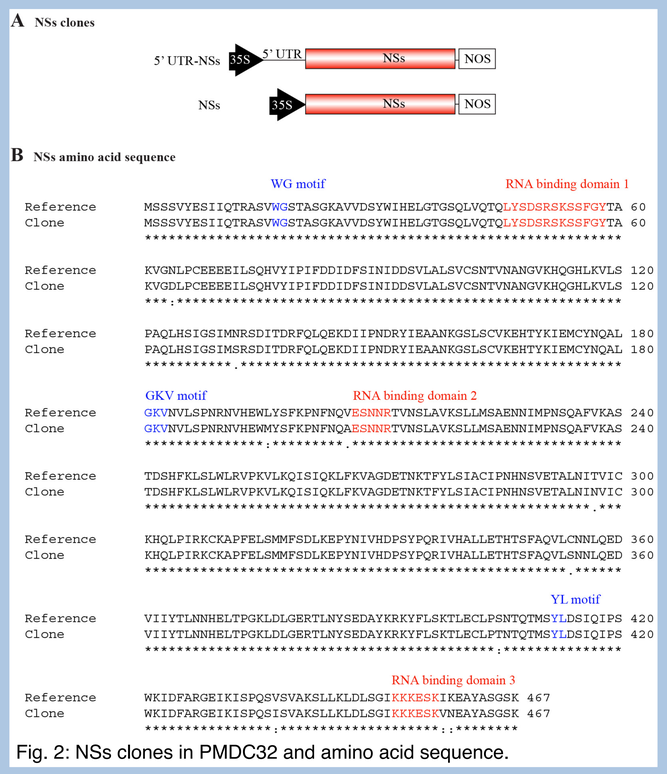

Fig. 2: NSs clones in PMDC32 and amino acid sequence.

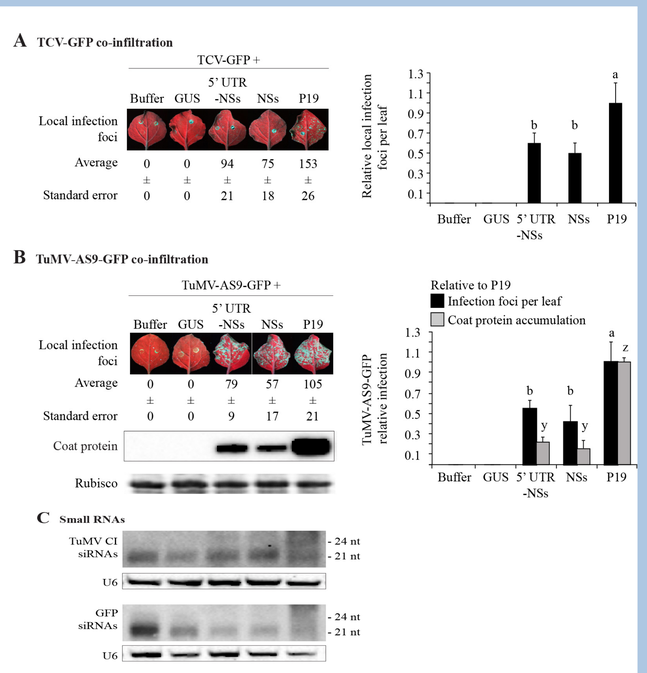

Fig. 3: NSs complements the pathogenicity defect in two suppressor-deficient viruses.

CONCLUSIONS

NSs Protein

- is a suppressor of antiviral RNA silencing.
- complements the pathogenicity defect in suppressor-deficient TuMV-AS9-GFP and in TCV-GFP.

TSWV:

- induces and suppresses RNA silencing in Arabidopsis.
- does not prevent the formation of virus-derived siRNAs.
- does not prevent the formation of endogenous siRNAs.
- does not trigger degradation of AGO1, AGO2, AGO10 proteins.

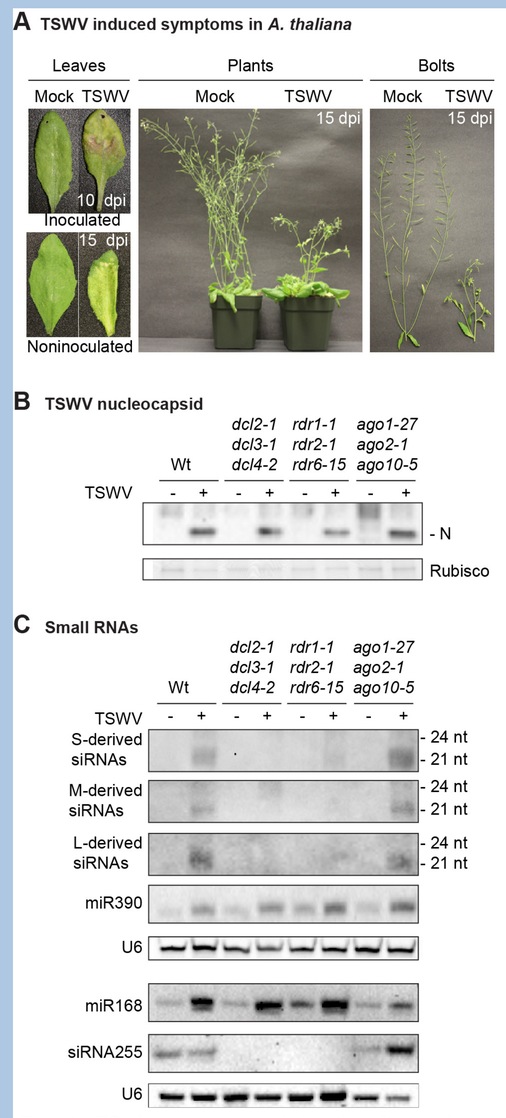

Fig. 4: TSWV is processed by the RNA silencing pathway in *Arabidopsis*. **A**. Symptoms after mechanical inoculation. Total protein and RNA were extracted at 15 dpi. **B**. TSWV infection was confirmed by immunoblotting against N protein. **C**. Accumulation of host- and virus-derived sRNAs was determined by northern blotting.

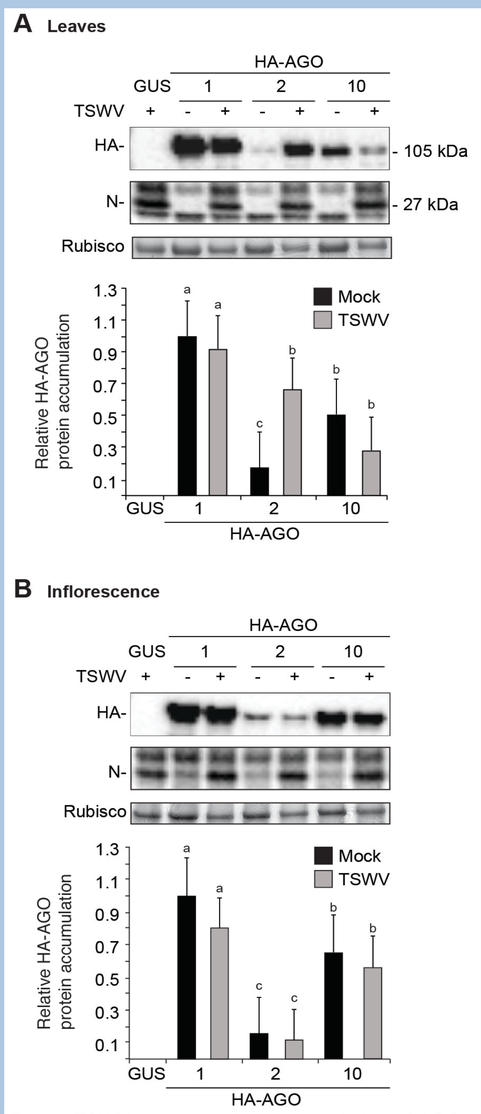

Fig. 5: TSWV does not trigger degradation of AGO1, AGO2, or AGO10. Total protein was extracted from virus-free or TSWV-infected transgenic *Arabidopsis* expressing HA-tagged AGO proteins. HA-AGO protein and TSWV accumulation were determined by immunoblotting.

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