

Synthesis of complementary RNA by RNA-dependent RNA polymerases in plant extracts is independent of an RNA primer

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Accessory publication

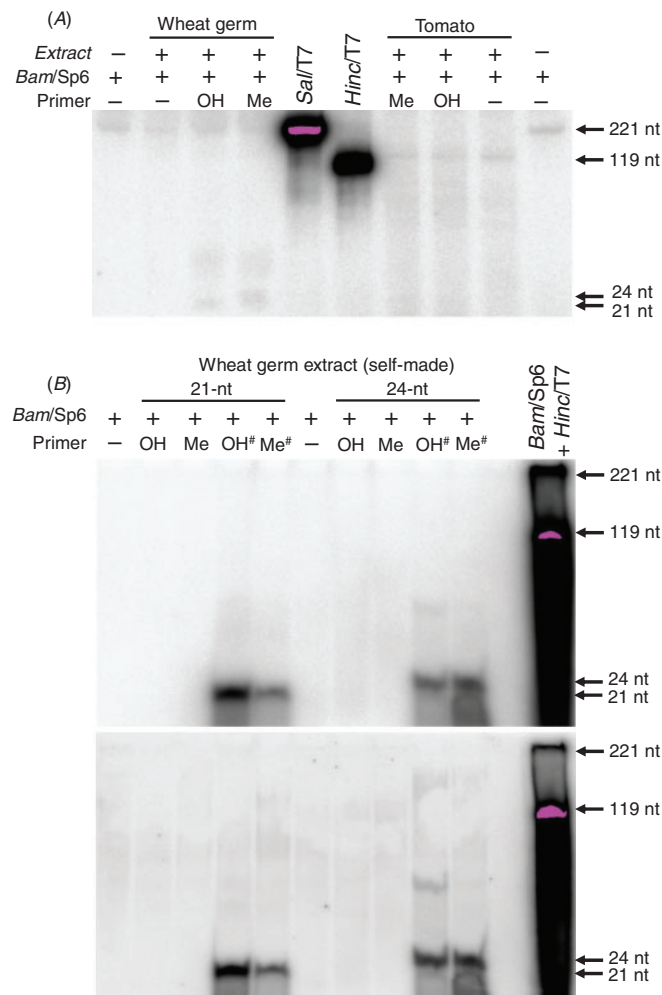


Fig. S1. No RDR activities could be detected in wheat germ and tomato leaf extracts. (A) RDR assays using wheat germ extract (purchased from Promega) and tomato leaf extract. The faint ~221-nt band was likely due to small amounts of contaminating antisense GUS RNA generated during *in vitro* transcription of *Bam*HI-linearized pMBW448 with Sp6 polymerase, as it was detected in control samples without the plant extracts (the first and last lanes). (B) RDR assay using unlabelled or radiolabelled (with the symbol #) RNA primers and wheat germ extract self-prepared according to Tang *et al.* (2003). The upper panel is a direct exposure of the gel blot while the lower panel is from hybridization of the same gel blot with ³²P-labelled *Bam*/Sp6 RNA probe to detect primer-independent product.

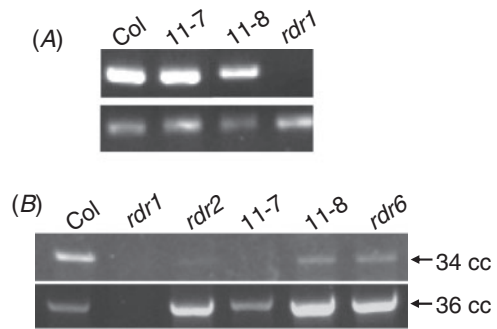


Fig. S2. (A) PCR-genotyping of RDR1 T-DNA insertion mutants. Primers used were specific for the Salk_112300 line. Absence of band indicates homozygous T-DNA insertion. Plants 11-7 and 11-8 were siblings from another Salk line of RDR1 mutation (Salk_025341). (B) RT-PCR detection of RDR1 transcript. Note that no RDR1 transcript was detected in the homozygous *rdr1* mutant, indicating complete loss of RDR1 expression. Plant 11-7 and 11-8 still expressed RDR1 so were likely heterozygous for the T-DNA insertion.