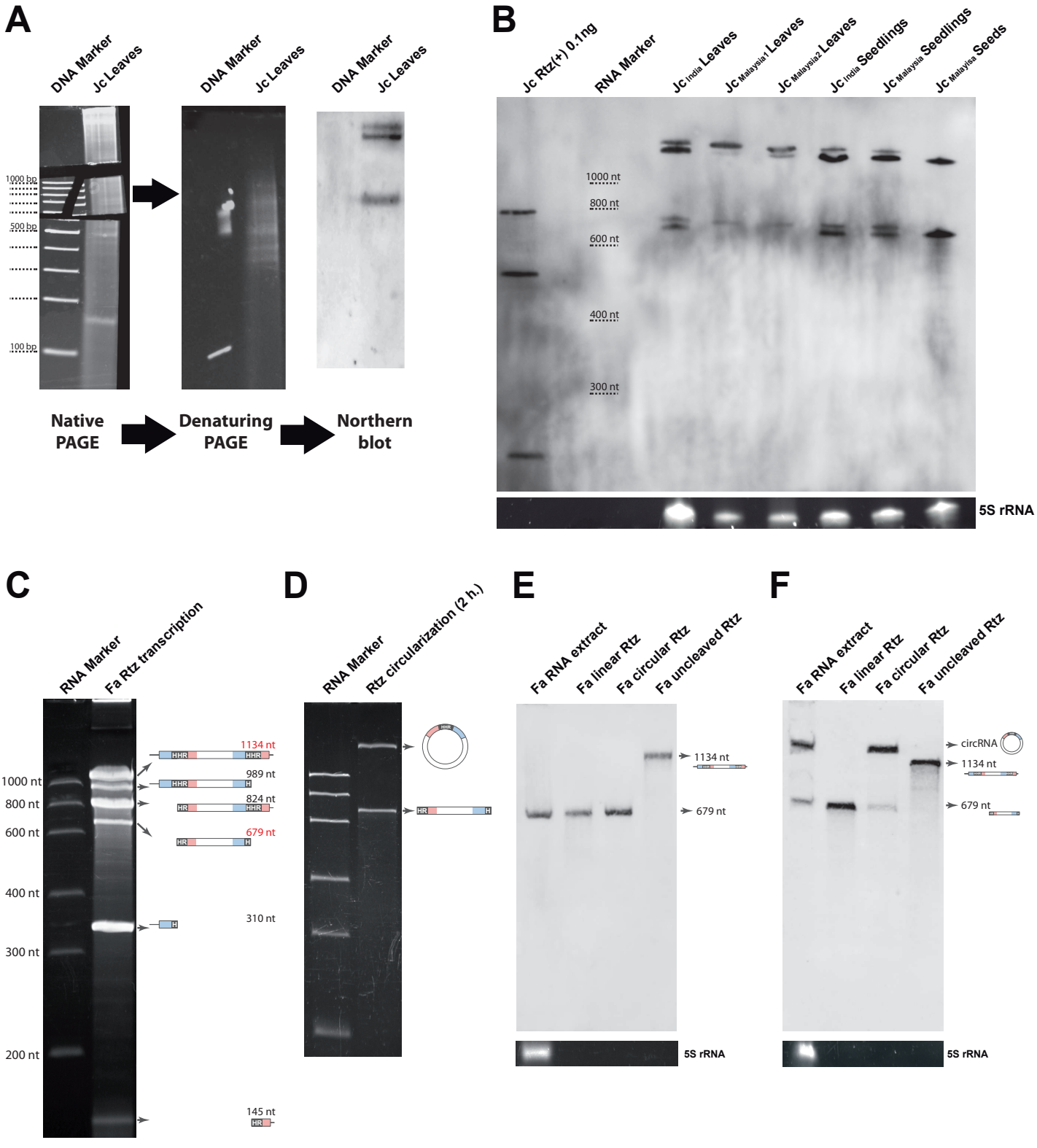

Additional file 5

Additional file 5.

Circular and linear retrozyme RNAs in *J. curcas* and *F. ananassa*. **a** Double PAGE analysis of an RNA extract (~20 μg) from *J. curcas* leaves. A gel stripe from a 5% native polyacrylamide gel containing RNAs of 600-900 nt (left) was cut out and run on top of a second 5% denaturing polyacrylamide gel (center). The corresponding Northern blot (right), using a digoxigenin-labelled *J. curcas* retrozyme fragment as a probe, revealed the presence of both circular and linear RNA forms. **b** Northern blot analysis of RNA extracts (~30 μg each) from *J. curcas* leaves, young seedlings and seeds. Samples were run on a 5% denaturing PAGE and were detected using the same probe as in panel 5a. **c** An *in vitro* transcription of a previously cloned full genomic retrozyme of *F. ananassa* was run on a 5% denaturing PAGE. The RNAs corresponding to the uncleaved full RNA (1,134 nt) and double self-cleaved retrozyme RNA (679 nt) were cut out and purified (marked in red). **d** Purified retrozyme RNA (679 nt) was circularized with a tRNA ligase for 2h and run on a 5% denaturing PAGE. The circularized RNA (upper band) was cut out and purified. **e** Northern blot hybridization of a *F. ananassa* RNA extract (~30 μg) and previously purified RNA markers (linear, circular and uncleaved full retrozyme RNAs, ~1 ng each) run on a 5% native PAGE. **f** Northern blot hybridization of the same RNA samples as in panel 5e run on a 5% denaturing PAGE. Ethidium bromide staining of the 5S rRNA is shown at the bottom as a loading control.