LETTER

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MicroRNAs from the parasitic plant *Cuscuta campestris* target host messenger RNAs

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Dodders (*Cuscuta* spp.) are obligate parasitic plants that obtain water and nutrients from the stems of host plants via specialized feeding structures called haustoria. Dodder haustoria facilitate bidirectional movement of viruses, proteins and mRNAs between host and parasite[1], but the functional effects of these movements are not known. Here we show that *Cuscuta campestris* haustoria accumulate high levels of many novel microRNAs (miRNAs) while parasitizing *Arabidopsis thaliana*. Many of these miRNAs are 22 nucleotides in length. Plant miRNAs of this length are uncommon, and are associated with amplification of target silencing through secondary short interfering RNA (siRNA) production[2]. Several *A. thaliana* mRNAs are targeted by 22-nucleotide *C. campestris* miRNAs during parasitism, resulting in mRNA cleavage, secondary siRNA production, and decreased mRNA accumulation. Hosts with mutations in two of the loci that encode target mRNAs supported significantly higher growth of *C. campestris*. The same miRNAs that are expressed and active when *C. campestris* parasitizes *A. thaliana* are also expressed and active when it infects *Nicotiana benthamiana*. Homologues of target mRNAs from many other plant species also contain the predicted target sites for the induced *C. campestris* miRNAs. These data show that *C. campestris* miRNAs act as trans-species regulators of host-gene expression, and suggest that they may act as virulence factors during parasitism.

In host-induced gene silencing (HIGS), siRNA-producing transgenes silence targeted pathogen and parasite mRNAs in *trans*[3,4]. Plant-based HIGS is effective against fungi[5], nematodes[6], insects[7] and the parasitic plant *Cuscuta pentagona*[8]. The ease with which HIGS can be introduced into plants suggests that they might exchange naturally occurring small RNAs with parasites. Consistent with this hypothesis, small RNAs from the plant pathogenic fungus *Botrytis cinerea* target host mRNAs during infection[9], and HIGS targeting of dicer-like mRNAs in *B. cinerea* reduces pathogen virulence[10]. Conversely, host miRNAs are exported from cotton into the fungal pathogen *Verticillium dahliae*[11]. However, to our knowledge, no examples of naturally occurring trans-species miRNAs have been described for plant–plant interactions.

Cuscuta haustoria facilitate bidirectional movement of viruses, proteins, and mRNAs[1], but the functional effects of these movements are unclear. *Cuscuta* is susceptible to HIGS, so we hypothesized that naturally occurring small RNAs might be exchanged across the *C. campestris* haustorium and affect gene expression in the recipient species. We profiled small-RNA expression in *C. campestris* grown on *A. thaliana* hosts using high-throughput small-RNA sequencing (small-RNA-seq). Two biological replicates each from three tissues were analysed: parasite stem, comprising a section of *C. campestris* stem above the site of haustorium formation; interface, comprising *C. campestris* stem with haustoria with associated *A. thaliana* stem tissue; and host stem, comprising sections of *A. thaliana* stems above the interface region, as previously described[12]. Small-RNA-producing loci from both organisms were identified, classified, and subjected to differential-expression analyses (Supplementary Data 1).

As expected, owing to dilution of parasite RNA with host RNA, *C. campestris* small-RNA loci were generally downregulated in the interface relative to the parasite stem (Fig. 1a). However, 76 *C. campestris* small-RNA species were significantly upregulated in the interface relative to the parasite stem (false discovery rate (FDR) $\leq$0.05). Of these interface-enriched species, 43 (57%) were miRNA species with canonical accumulation of discrete miRNA–miRNA* pairs (the expected processing intermediates of miRNA biogenesis) from

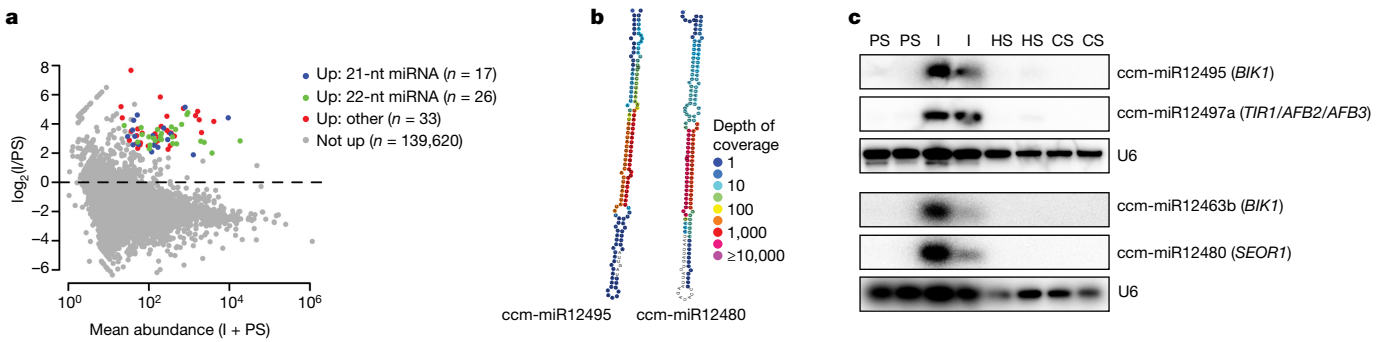

Figure 1 | *C. campestris* miRNAs induced at the haustorial interface. **a**, Mean abundance plot of *C. campestris* small-RNA loci comparing interface (I) to parasite stem (PS). Significantly upregulated (Up) loci are highlighted (alternative hypothesis: true difference > 2-fold, FDR $\leq$ 0.05 after Benjamini–Hochberg correction for multiple testing). nt, nucleotide. **b**, Predicted secondary structures of induced *C. campestris* miRNA hairpin precursors with colour-coded small-RNA-seq coverage per nucleotide. **c**, RNA blots of 22-nucleotide interface-induced miRNAs. HS, host stem; CS, control stem. U6, small nuclear RNA loading control. The experiment was performed twice with similar results. Full gels are shown in Supplementary Fig. 1.

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