predicted stem–loop precursors (Fig. 1b, Supplementary Data 2–4). RNA blots confirmed interface-specific expression of specific miRNAs (Fig. 1c). One of the 43 miRNAs is a member of the conserved *MIR164* family; the other 42 upregulated miRNAs have low sequence similarity to known miRNA loci, and none of the mature miRNAs or miRNA* aligned perfectly with the *A. thaliana* genome (Supplementary Data 5). Several of the miRNA loci were detected by PCR of *C. campestris* genomic DNA prepared from four-day old seedlings that had never interacted with a host plant (Extended Data Fig. 1). The majority of the induced *C. campestris* miRNAs (26 out of 43) produced a 22-nucleotide mature miRNA. Such 22-nucleotide miRNAs occur less frequently than 21-nucleotide miRNAs in plants, and they are strongly associated with accumulation of secondary siRNA from their targets[13,14]. Secondary siRNAs are thought to amplify miRNA-directed gene silencing[2].

We hypothesized that the induced 22-nucleotide miRNAs would cause formation of secondary siRNA from targeted host mRNAs. Therefore, we searched small-RNA-seq data for *A. thaliana* mRNAs that both contained plausible miRNA-complementary sites and shared sequences with siRNAs that accumulated specifically at the interface. Six *A. thaliana* mRNAs were found that met both criteria: *TIR1*, *AFB2* and *AFB3*, which encode partially redundant auxin receptors[15]; *BIK1*, which encodes a plasma-membrane-localized kinase required for both pathogen-induced and developmental signalling[16,17]; *SEOR1*, which encodes an abundant phloem protein that reduces photosynthate loss from the phloem after injury[18,19]; and *HSFB4* (also known as *SCZ*), which encodes a predicted transcriptional repressor that is required for the formation of ground-tissue stem cells in roots[20–22]. The siRNAs produced from these mRNAs resembled other examples of secondary siRNAs in their size distributions, double-stranded accumulation, and phasing (Fig. 2a, b, Extended Data Fig. 2). *TIR1*, *AFB2* and *AFB3* are also known to be targeted by the 22-nucleotide miRNA miR393, and to produce secondary siRNAs downstream of the miR393-complementary site[23]. In parasitized stems, the location and phase register of the *TIR1*, *AFB2* and *AFB3* secondary siRNAs shift upstream, proximal to the sites that are complementary to the *C. campestris* miRNAs (Extended Data Fig. 2), implying that the *C. campestris* miRNAs, and not miR393, are triggering the interface-specific secondary siRNAs. The predominant 21-nucleotide phase register at several loci was shifted by +1 to +2 nucleotides relative to predictions. This is consistent with the 'phase drift' seen at other phased siRNA loci[24,25] that cause the register to be shifted forward, and is probably due to the presence of low levels of 22-nucleotide siRNAs. Analysis of uncapped mRNA fragments showed strong evidence for miRNA-directed cleavage at all of the sites complementary to *C. campestris* miRNAs, specifically those from interface samples but not from control stem samples (Fig. 2, Extended Data Fig. 2). We did not find any induced miRNAs or siRNAs from the *A. thaliana* host that were capable of targeting these six mRNAs. We also did not find any endogenous *C. campestris* secondary siRNA loci corresponding to any of the induced miRNAs. Some *C. campestris* orthologues of *TIR1, HSFB4* and *BIK1* had possible, but very poorly complementary, miRNA target sites (Extended Data Fig. 3). These observations suggest that the induced *C. campestris* miRNAs have evolved to avoid targeting 'self' transcripts. We conclude that 22-nucleotide miRNAs from *C. campestris* act in a trans-species manner to target *A. thaliana* mRNAs.

Accumulation of five of the six targets was significantly reduced in parasitized stems compared to control stems (Fig. 3a). The true magnitude of repression of these targets could be even greater, since many miRNAs also direct translational repression. Accumulation of *A. thaliana* secondary siRNAs is partially dependent on the endonuclease DCL4 (DICER-LIKE 4) and wholly dependent on RDR6 (RNA-DEPENDENT RNA POLYMERASE 6, also known as SGS2 or SDE1)[2]. Accumulation of an abundant secondary siRNA from *TIR1* was eliminated entirely in the *sgs2-1* mutant, and reduced in the *dcl4-2t* mutant (Fig. 3b). Thus, host *DCL4* and *RDR6* are required for secondary siRNA production. This implies that the *C. campestris*-derived miRNAs are

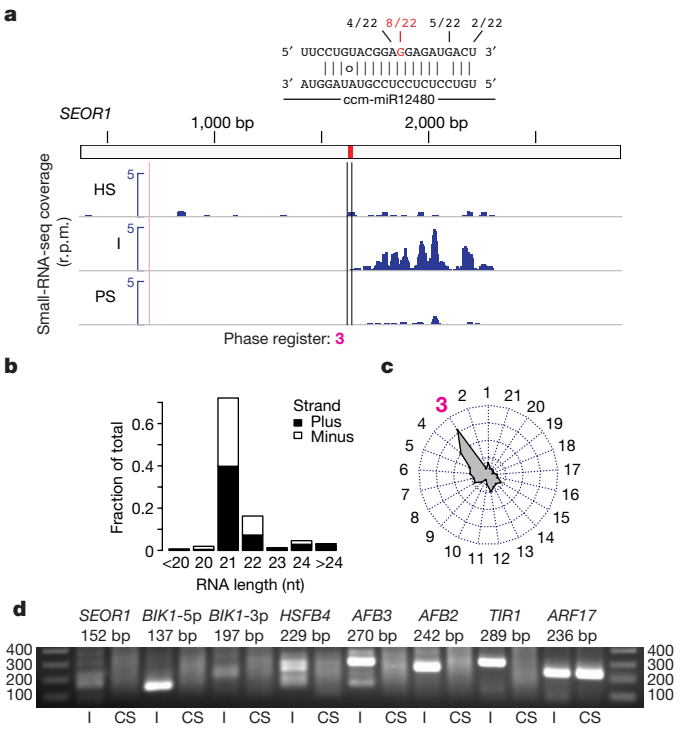

Figure 2 | *C. campestris* miRNAs cause slicing and phased siRNA production from host mRNAs. a, Small-RNA-seq coverage for *A. thaliana SEOR1* for host stem, interface and parasite stem. $n = 2$ biologically independent samples each; miRNA-complementarity is shown by RNA-ligase-mediated 5′-rapid amplification of cDNA ends (5′-RLM-RACE) and expected phase register; r.p.m., reads per million. **b**, Length and polarity distribution of *SEOR1*-mapped siRNAs from interface samples. **c**, Radar chart showing fraction of interface-derived siRNAs in each possible 21-nucleotide phasing register. **d**, 5′-RLM-RACE products from nested amplifications. *ARF17*, positive control. The experiment was performed once. Full gels are shown in Supplementary Fig. 1.

active inside host cells and hijack the host's own silencing machinery to produce secondary siRNAs.

In repeated trials, we did not observe consistent significant differences in parasite fresh weight using *dcl4-2t* and *sgs2-1* mutants as hosts (Extended Data Fig. 4). Thus, loss of induced secondary siRNAs is not sufficient to affect parasite growth in this assay. Similarly, there were no significant differences in biomass of *C. campestris* grown on *scz2* or *tir1-1/afb2-3*-mutant hosts (Fig. 3c). Significantly less ($P < 0.05$) *C. campestris* biomass was observed using the *bik1* mutant as host (Fig. 3c). However, interpretation of this result was complicated by the weak, frequently lodging stems of the *bik1* mutant[16]. *BIK1* is involved in both plant development and immunity, and its developmental functions may mask its role in the *C. campestris* interaction. Significantly more ($P < 0.05$) *C. campestris* biomass was observed on *seor1* or *afb3–4* mutants (Fig. 3c). Therefore, both *SEOR1* and *AFB3* function to restrict *C. campestris* growth on *A. thaliana*. This observation is consistent with the hypothesis that both *SEOR1* and *AFB3* are trans-species miRNA targets of biological relevance in *A. thaliana*.

C. campestris has a broad host range among eudicots[26]. Therefore, we searched for sites in eudicot orthologues of the targeted *A. thaliana* mRNAs that were complementary to the *C. campestris* miRNAs induced specifically at the interface. Probable orthologues of *BIK1, SEOR1, TIR1* and *HSFB4* were identified as predicted targets of interface-induced miRNAs in many eudicot species, while only one species had predicted targets in the orthologues of the negative control *GAPDH* (Fig. 4a, Extended Data Table 1). We conclude that the induced *C. campestris* miRNAs would be able to collectively target *TIR1, SEOR1, HSFB4* and *BIK1* orthologues in many eudicot species.