Long-distance transport of mRNA via parenchyma cells and phloem across the host–parasite junction in *Cuscuta*

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Summary

• It has been shown that the parasitic plant dodder (*Cuscuta pentagona*) establishes a continuous vascular system through which water and nutrients are drawn. Along with solutes, viruses and proteins, mRNA transcripts are transported from the host to the parasite. The path of the transcripts and their stability in the parasite have yet to be revealed.

• To discover the route of mRNA transportation, the *in situ* reverse transcriptase–polymerase chain reaction (RT-PCR) technique was used to locally amplify host transcript within parasitic tissue. The stability of host mRNA molecules was also checked by monitoring specific transcripts along the growing dodder thread.

• Four mRNAs, α and β subunits of *PYROPHOSPHATE* (*PPi*)*-DEPENDENT PHOSPHOFRUCTOKINASE* (*LePFP*), the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), and *GIBBERELLIC ACID INSENSITIVE* (*LeGAI*), were found to move from host (tomato (*Solanum lycopersicum*)) to dodder. *LePFP* mRNA was localized to the dodder parenchyma cells and to the phloem. *LePFP* transcripts were found in the growing dodder stem up to 30 cm from the tomato–dodder connection.

• These results suggest that mRNA molecules are transferred from host to parasite via symplastic connections between parenchyma cells, move towards the phloem, and are stable for a long distance in the parasite. This may allow developmental coordination between the parasite and its host.

Key words: *Cuscuta*, dodder, *in situ* RT-PCR, long-distance movement, mRNA trafficking, parasite, phloem.

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Introduction

Parasitism among plants is a fascinating phenomenon in which one plant establishes a nonmutual dependence on another. Parasitic plants have evolved at least 11 times among the angiosperms (Barkman *et al.*, 2007) and are distributed among 17 families (Parker & Richard, 1993). Consistent with multiple origins of parasitism, a wide range of parasitic strategies can be observed, varying from obligate endoparasitism to facultative hemiparasitism. Among the most common parasitic plants are *Cuscuta*, *Orobanche*, *Striga*, *Viscum* and *Cassytha* (Moreno *et al.*, 1997). These plants establish a connection with their host by growth of haustorial structures. The haustorium is used to connect the parasite vascular system to the host vascular system, allowing transfer of the vascular contents from the host to the parasite (Dawson *et al.*, 1994). Consequently, parasitism retards normal plant growth and causes substantial crop losses in many economically important crops in both the developed and the developing world (Dawson *et al.*, 1994).

Carbohydrate acquisition at some stage of growth is a requirement for all obligate parasitic plants (Hibberd & Jeschke, 2001). In several parasitic weeds, the nature of the connection has been determined to be symplastic. The establishment of this interaction involves the formation of intricate connections

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between the parasite and its host (Dorr, 1996; Vaughn, 2003). Such interspecific plasmodesmata have been observed in root parasites such as *Striga* and *Orobanche* (Dorr & Kollmann, 1995; Dorr, 1996) as well as in the obligate parasite *Cuscuta* (dodder) (Dawson *et al.*, 1994; Vaughn, 2003), and allow the parasite access to the symplastic contents of the hosts.

The genus *Cuscuta* consists of *c.* 150 species that parasitize the above-ground tissues of both monocots and dicots worldwide (Malik & Singh, 1979; Dawson *et al.*, 1994; Lanini & Kogan, 2005). Dodder is an agriculturally destructive weed that causes serious damage by suppressing the growth of its host, in some cases leading to host death (Dawson *et al.*, 1994). Dodder is prolific at seed setting and the seeds can remain dormant for 5 yr or more. Dodder seeds germinate and form a thread-like stem with a small root. The seed lacks enough reserves for sustained seedling growth. The seedling contains little chlorophyll, and for survival is completely dependent on finding a host within a few days of germination (Hibberd *et al.*, 1998). Upon contact with a compatible host, the dodder hypha (hairlike filament) grows into the host cells and induces synthesis of a unique cell wall that encases the invading hyphae, which then differentiate to form the haustorium (Vaughn, 2003). The haustorium penetrates the host plant and continues to form 'searching hyphae' that grow toward the host vascular system (Dawson *et al.*, 1994; Vaughn, 2003). Many plasmodesmata are formed at the tip of the searching hyphae where a point of contact is created with the host parenchyma cells (Vaughn, 2003; Birschwilks *et al.*, 2006). Simultaneously, parenchyma cells in the haustorium differentiate into xylem and phloem elements that associate with the host vasculature (Tsivion, 1978; Jeschke *et al.*, 1994). Eventually, phloem–phloem and xylem–xylem connections between dodder and the host are formed through which water and assimilates are transferred to the parasite (Tsivion, 1978; Vaughn, 2003; Birschwilks *et al.*, 2006).

The continuous host–dodder vascular connection was demonstrated by the transfer of various molecules from host to dodder phloem. For instance, sugars were shown to be transported from an alfalfa (*Medicago sativa*) host into dodder in radioactive feeding experiments (Tsivion, 1978). Furthermore, radiolabeled amino acids were shown to move from host to parasite (Birschwilks *et al.*, 2006). Green fluorescent protein (GFP) has been shown to cross the host–dodder junction in transgenic *Nicotiana tabacum* plants parasitized by *Cuscuta reflexa* (Haupt *et al.*, 2001). GFP was also used to visualize the translocation of the tobacco mosaic virus movement protein from *N. tabacum* parenchyma cells to the searching hypha of *C. reflexa* (Birschwilks *et al.*, 2006). Fluorescent probes that were applied to *N. tabacum* leaves were shown to be present in the dodder vascular system (Haupt *et al.*, 2001; Birschwilks *et al.*, 2006). Recently, it was demonstrated that there is a peripheral size exclusion limit for protein movement from Arabidopsis transgenic plants to dodder (Birschwilks *et al.*, 2007). In this study it was shown that GFP but not GFP-ubiquitin is translocated to dodder through the phloem. The same study also demonstrated the movement of GFP from transgenic Arabidopsis to a wild-type plant via a dodder bridge in a source-to-sink dependent manner (Birschwilks *et al.*, 2007). In addition, virus movement from one host to another via a dodder bridge has been reported (Hiosford, 1967; Birschwilks *et al.*, 2006). More recently, phloem mobile mRNAs have been shown to traffic between tomato (*Solanum lycopersicum*) and dodder (Roney *et al.*, 2007). It was not shown whether these molecules reach the parasite through direct phloem–phloem connections or parenchyma cell connections. Additionally, it is not known whether host mRNA molecules are stable in the parasite over a long distance.

In this study, we show that intact mRNA molecules are transferred from two different hosts, tomato and alfalfa, into the parasite dodder. We demonstrate that the host mRNA is localized to the dodder parenchyma cells as well as to sieve elements near the point of contact with the host. Furthermore, we show that these molecules are stable for physiologically relevant distances, implying a role for these molecules in dodder–host relationships.

Materials and Methods

Plant material

Dodder (*Cuscuta pentagona* Engelmann) was germinated from seed and grown on the host plant *Oxalis* sp. until dodder tendrils began to grow out. Tomato (*Solanum lycopersicum* L. cv.VF36) and alfalfa (*Medicago sativa* L.) plants were grown from seeds planted directly into soil. Dodder tendrils were then placed on young tomato and alfalfa plants approx. 4 wk after germination. Successful infection took place approx. 2 wk later, at which point the tendrils were cut from *Oxalis* sp. All plant material was grown in a glasshouse (23°C and 50% humidity) at the University of California, Davis (CA, USA).

Histology

Tissues were fixed in FAA (formaldehyde 1.85%, glacial acetic acid 5%, and ethanol 63%), dehydrated through an ethanol series (70, 80, 90 and 100% for 30 min each), embedded in paraffin, sectioned using a Microtome HM 340 E (Microm International, Walldorf, Germany), and stained with 0.1% toluidine blue O. The sectioned material was observed using a Nikon Eclipse E600 microscope and digital images were taken using a SPOT RT camera (Diagnostic Instrument, Sterling Heights, MI, USA). Photographs of tomato plants parasitized by dodder were taken using an Olympus SP-500UZ digital camera or via the Zeiss Discovery V12 dissecting microscope and Zeiss AxioCAm MRc digital camera.

Scanning electron microscopy (SEM) analysis

Tissue of dodder connected to tomato stem and leaf was fixed as described previously (Bharathan *et al.*, 2002). Electronic images

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were obtained with a Hitachi S-3500 N scanning electron microscope (SEM; Hitachi Science Systems Ltd, Tokyo, Japan).

Cloning the dodder *PYROPHOSPHATE (PPi)-DEPENDENT PHOSPHOFRUCTOKINASE* (*CpPFP*) β subunit

The dodder *CpPFP* β subunit was cloned by PCR using degenerate primers designed to span conserved regions in an alignment of *PFP* sequences from multiple species. Primer sequences were: dePFP145F: 5′-GGACGNNTNGCNTCCGTTTACAGC-GAAGTCC-3′;
dePFP766R: 5′-GGAACCTCTTTGCATTTCAANTCAC-CATC-3′.

PCR conditions were as follows: 2 min at 94°C; 40 cycles of 20 s at 94°C, 20 s at 46°C and 60 s at 72°C, terminated by 10 min at 72°C. The PCR band was eluted using the QIAquick Gel Extraction Kit (Qiagen Inc, Germantown, MD, USA) and cloned into Topo 2.1 (Invitrogen, Carlsbad, CA, USA) for sequencing. Sequencing was performed by Davis Sequencing (Davis, CA, USA). The dodder *PFP* β subunit identity was confirmed using the BLAST program at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov). The *C. pentagona PFP* β subunit sequence was deposited in Genbank and given the accession number DQ858165.

Reverse transcriptase–polymerase chain reaction (RT-PCR)

Following successful infection, 3–4-cm dodder stems were harvested 1 cm from the host junction avoiding tissue in direct contact with the host. Total RNA extraction was performed with the Qiagen RNeasy kit (Qiagen Inc.) according to the manufacturer's protocol. cDNA was synthesized from 250 ng of total RNA using Superscript III (Invitrogen) after RQ1 RNase Free DNAse treatment (Promega, Madison, WI, USA). Random hexamers were used for all RT reactions. RNA was quantified using the NanoDrop ND1000 model spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). PCR conditions were as follows: for *LePFP* β and *LePFP* α: 2 min at 94°C and 50 cycles of 20 s at 94°C, 20 s at 53°C and 60 s at 72°C, terminated by 10 min at 72°C; for *CpPFP*, *GIBBERELLIC ACID INSENSITIVE* (*LeGAI*) and *RUBISCO SMALL SUBUNIT* (*RbcS*): 2 min at 94°C and 52 cycles of 20 s at 94°C, 20 s at 53°C and 60 s at 72°C, terminated by 10 min at 72°C; for *MtPFPα*: 2 min at 94°C and 50 cycles of 20 s at 94°C, 20 s at 52°C and 60 s at 72°C, terminated by 10 min at 72°C. To verify the identity of the PCR bands, amplification products were directly sequenced with the forward primer used in the reaction and aligned to host sequences using VECTOR NTI ADVANCE software (Invitrogen). At least two independent biological replicates were employed for each experiment.

The primers for the *S. lycopersicum PFP* α subunit (accession number: AW216363 + AW035056) were: LePFPaF, 5′-GAGATCCACACGGCAATGTCCAGGTTGCTA-3′ and LePFPaR, 5′-CACTGGCTTGAACTTGCCATGTCTCCTCTC-3′. The primers for the *S. lycopersicum PFP* β subunit (accession number: BT012702) were: LePFPbF, 5′-AATGAACTCCG-GGGTCAACC-3′ and LePFPbR, 5′-TCGTTGATGGTC-CTCCTTCA-3′. The primers for the *S. lycopersicum* GAI-like protein-encoding gene (accession number: AY269087) were: LeGAIF, 5′-AGTCGTCTGATATGGCGGATG-3′ and LeGAIR, 5′-CGGTGAGTCTAAATGCCGGAGGT-3′. The primers for *S. lycopersicum RbcS* (accession number: LERBCS1) were: LeRBCsF, 5′-TGTTGCTCAAGCTAGCATGGTCG-CAC-3′ and LeRBCsR, 5′-TTGGAAATTTAGAATCCT-TCTG-3′. The primers for alfalfa were designed based on *M. truncatula* sequences. For the *PFP* α subunit (accession numbers: BI270737 and AW267791), the primers were: MtPFPaF, 5′-TGCTCTCTCTGCAGAGAAGTA-3′ and MtP-FPaR, 5′-AACCTCCTCACCAAGAATTACCATGTTTGG-3′. The primers for the *C. pentagona PFP* β subunit (accession number: DQ858165) were: CpPFPbF, 5′-TTGTCGACG-GCCCCGCTAGC-3′ and CpPFPbR, 5′-CTCCACCGAT-CACAACAAGC-3′.

In situ RT-PCR

Tissue of dodder attaching to tomato or alfalfa stem or leaf was fixed in FAA for *in situ* RT-PCR (formaldehyde 1.85%, glacial acetic acid 5%, and ethanol 63%), embedded in paraffin and sectioned using a Zeiss Microtome HM340E. Slides were treated as previously described by Long *et al.* (1996). The RT-PCR step was performed (instead of probe hybridization) following the protocol of Ruiz-Medrano *et al.* (1999) as described. The following primers were used in this experiment:
LePFPf: 5′-GGTTTTGACACAATATGCAAGGTGAAC-3′ and
LePFPr: 5′-GATAGCAGACGTGACCAAGTACATAT-GCATA-3′.

These primers were designed to span the exon–exon junctions to specifically amplify cDNA of the tomato *PFP* α subunit, resulting in a 664-bp fragment. Anti-Dig antibodies conjugated to alkaline phosphatase were used to detect Dig-labeled cDNA molecules. Two independent biological replicates were employed for each experiment. Signal was detected using the Nikon Eclipse E600 microscope and digital images were collected using a SPOT RT camera (Diagnostic Instrument). Nomarski optics was utilized to amplify the image contrast of the alfalfa negative control, where no signal was detected.

Results

Establishment of host–dodder connection

In order to visualize mRNA movement from host to parasite, establishment of a host–dodder connection was confirmed. *Solanum lycopersicum* (tomato) and *M. sativa* (alfalfa) were used

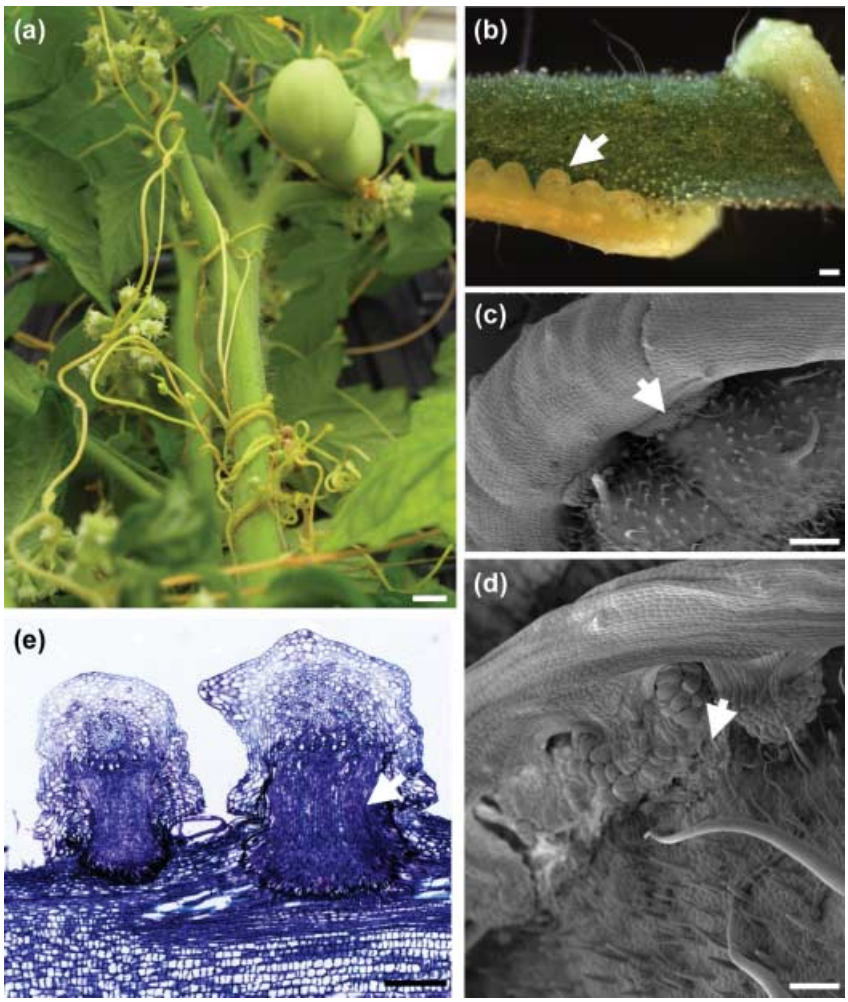

Fig. 1 Parasite–host interaction of tomato (*Solanum lycopersicum*) and dodder (*Cuscuta pentagona*). (a) Dodder parasitizing a 7-wk-old tomato plant, 4 wk after attachment. Bar, 5 mm. (b) Haustorium formation on tomato petiole (arrow). Bar, 500 μm. (c) Scanning electron microscope (SEM) image of young haustoria (arrow) in dodder–tomato interaction. (d) SEM image of mature haustoria (arrow) detaching from tomato leaf demonstrating the interactions between the two organisms. (e) A cross-section of two adjacent haustoria establishing an internal connection (arrow) with the tomato host leaving a penetration fissure behind. Bar, 500 μm.

as hosts for the *C. pentagona* (dodder) parasite. The interaction of dodder with the tomato host was evident after 6–8 d, at which point attachment occurred. Growth of the dodder stem continued until the host was fully infected at 3 wk (Fig. 1a). Despite multiple haustorial connections on the stems, petioles and leaves, the tomato host continued to grow through to fruit setting (Fig. 1a,b). The haustorium structure includes elongated cells that are growing toward the host stem and leaf (Fig. 1b,c, arrows). SEM analysis revealed the formation of haustoria soon after the dodder contacted the host (Fig. 1c, arrow), which later developed into mature haustoria (Fig. 1d, arrow). Histological sections of mature huastoria revealed an internal connection between the parasite and the host (Fig. 1e, arrow), which eventually reached the vasculature system of the host (Vaughn, 2003). In addition to tomato, alfalfa was selected as the second species for these experiments, not only because of its susceptibility to dodder but because it represents a host that is more distantly related to dodder than tomato. The alfalfa–dodder connections were similar to those seen in tomato–dodder interactions (data not shown).

Tomato and alfalfa mRNA transcripts move into dodder

Both tomato–dodder and alfalfa–dodder systems were used for detecting mRNA movement from host to parasite. For further analysis, four different gene transcripts were chosen based on previous studies that showed their long-distance movement capability in tomato. *PFP* has been shown to move long distances via the phloem in tomato (Kim *et al.*, 2001). Thus, transcripts of the tomato *PFP* α and β subunits were chosen for RT-PCR analysis. In addition, the tomato transcript *LeGAI* was shown to travel long distances via the phloem, to reach the shoot apical meristem and to affect leaf development (Haywood *et al.*, 2005). The fourth transcript, the tomato small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO; *Rbc*S), was selected because it is a nuclear gene with a high level of transcription in tomato, although it was not detectable in phloem sap of one other species (pumpkin (*Cucurbita maxima*)) (Haywood *et al.*, 2005).

Primers that were used to specifically amplify the tomato *LePFP* α and β subunits supported amplification of these

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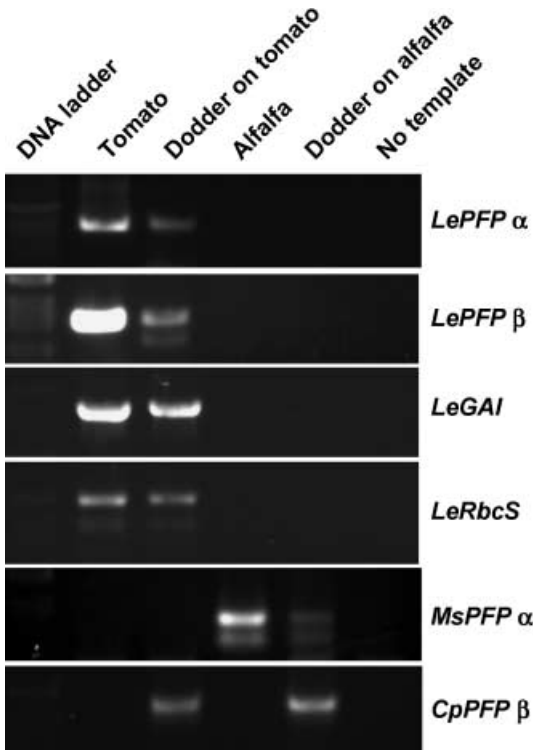

Fig. 2 Host transcript amplification from dodder (*Cuscuta pentagona*) cDNA for multiple genes. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis was performed on RNA isolated from host tissue and dodder grown on the host. Amplification of tomato (*Solanum lycopersicum*) transcripts *PYROPHOSPHATE* (*PPi*)*-DEPENDENT PHOSPHOFRUCTOKINASE* (*LePFP*) α and β, *GIBBERELLIC ACID INSENSITIVE* (*LeGAI*) and *RUBISCO SMALL SUBUNIT* (*LeRbcS*) is evident in cDNA made from tomato and dodder grown on tomato but not alfalfa (*Medicago sativa*) or dodder grown on alfalfa. *MsPFP* α transcript amplification is evident in alfalfa and dodder grown on alfalfa but not in tomato or dodder grown on tomato. Dodder *PFP* (*CpPFP*) β transcript amplification occurs only in cDNAs made from dodder and not in either host (lowest panel). At least two independent biological replicates were employed for each experiment. Negative control was a PCR reaction without a template (no template).

transcripts from cDNA made from tomato and from dodder plants grown on tomato (Fig. 2). However, these primers showed no amplification from cDNA made from alfalfa or dodder grown on alfalfa (Fig. 2).

Similar results were observed for *LeGAI*, where its transcript was found in tomato and dodder grown on tomato, but not in alfalfa or dodder grown on alfalfa (Fig. 2). These results are in agreement with the recently reported presence of *LeGAI* transcript in dodder grown on tomato (Roney *et al.*, 2007). Similarly, the *LeRbc*S transcript was found to be present in tomato and in dodder grown on tomato, but not in alfalfa or dodder grown on alfalfa (Fig. 2). These results show movement of mRNA transcripts from the tomato host to the dodder parasite.

A similar experiment was conducted for the interaction between dodder and alfalfa. Again, primers specific to the α subunit of *M. truncatula PFP* (*MtPFP*) were used to detect the presence of this transcript in dodder grown on alfalfa (*M. sativa*; Fig. 2). Amplification of the α subunit of *MsPFP* was detected in cDNA made from alfalfa and dodder grown on alfalfa, whereas amplification was not supported from cDNA made from tomato or dodder grown on tomato (Fig. 2).

In order to determine whether dodder transcripts move into the host, the dodder *PFP* (*CpPFP*) β subunit was cloned. Primers designed to specifically amplify the dodder *PFP* β subunit supported amplification from cDNA made from dodder grown on tomato and dodder grown on alfalfa. However, by RT-PCR analysis, these primers failed to amplify dodder *PFP* from cDNA made from tomato or alfalfa parasitized by dodder (Fig. 2).

The host mRNA in dodder is localized to parenchyma cells and phloem

To further analyze the mRNA translocation from host to dodder and determine what path mRNA takes, *in situ* RT-PCR experiments on fixed sections of dodder on tomato and dodder on alfalfa were carried out. This experiment was designed to specifically amplify the tomato *PFP* α transcript. The primers used for these experiments were designed to span exon–exon junctions and were found to successfully amplify cDNA while failing to amplify product from tomato genomic DNA (data not shown).

The results from *in situ* RT-PCR revealed a strong signal in tomato parenchyma cells but not in the epidermal layer (Fig. 3a). A strong signal was also present in the dodder parenchyma cells but absent in the epidermal layer (Fig. 3b). The strongest signal in dodder tissue was localized to parenchyma cells that were closer to the host (Fig. 3c) and no signal was detected in the xylem or parenchyma cells opposite the haustorium (Fig. 3c). Close examination revealed that the signal was localized to the region corresponding to the sieve tube elements and companion cells (Fig. 3d) as observed in toluidine blue O-stained sections (Fig. 3f). No signal was detected in alfalfa or dodder tissue parasitizing alfalfa (Fig. 3e). These results are consistent with those of the RT-PCR experiments using cDNA and signify the specificity of the primers used in this experiment. Taken together, these results suggest that mRNA translocation from host to parasite occurs through parenchyma cells to the phloem.

Host transcript can be detected at large distances from the host junction

In attempts to reveal whether host gene transcripts are stable for a long distance in the parasite, mRNA was analyzed along the dodder growing thread. RT-PCR analysis was performed on RNA extracted from segments at 5-cm intervals up to 64 cm from the tomato–dodder junction on actively growing strands. As expected, high expression of the *LePFP* β subunit was evident in tomato tissue (Fig. 4). The *LePFP* β transcript was also detectable in dodder up to 20 cm from the tomato–dodder junction in one biological replicate (Fig. 4) and up to 30 cm

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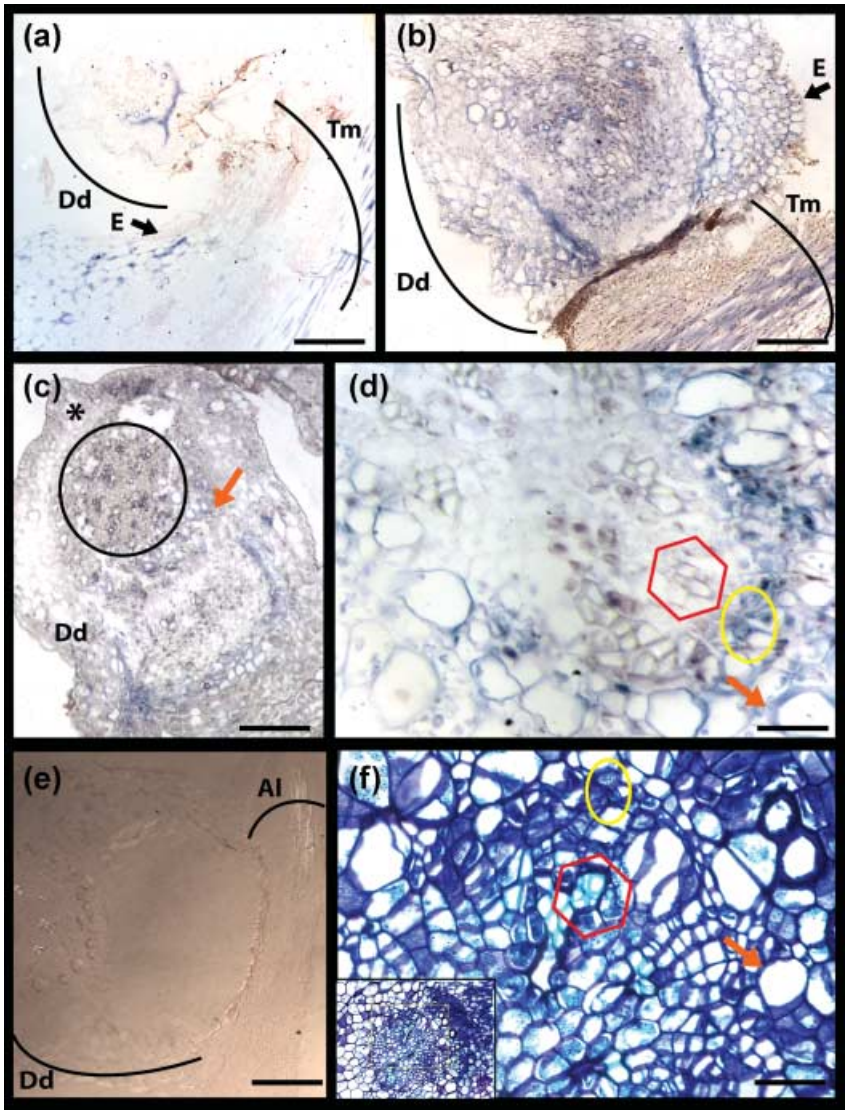

Fig. 3 Light microscope images of *in situ* reverse transcriptase–polymerase chain reaction (RT-PCR) analysis of the tomato (*Solanum lycopersicum*) *PYROPHOSPHATE* (*PPi*)*-DEPENDENT PHOSPHOFRUCTOKINASE* (*LePFP*) α subunit. (a, b) Purple-blue signal indicating expression of the *LePFP* α subunit in dodder (*Cuscuta pentagona*) (transverse section) and in tomato tissue (longitudinal section). Dd, dodder; Tm, tomato; E, epidermis. (c) Expression of the *LePFP* α subunit in dodder parenchyma cells closer to the host (orange arrow). The vascular cylinder is enclosed in a black circle. The star indicates the region opposite the haustorium. (d) A close-up of the vascular cylinder showing expression of the *LePFP* α subunit (purple-blue signal) in dodder phloem tissue (yellow oval) and parenchyma cells (orange arrow) but not in xylem tissue (red hexagon). (e) Nomarski contrast image showing no signal in a cross-section of alfalfa–dodder interactions. Dd, dodder; Al, alfalfa (*Medicago sativa*). (f) A cross-section of the dodder vascular cylinder, analogous to (d), stained with toluidine blue O, showing sieve elements and companion cells (yellow oval), xylem (red hexagon) and parenchyma cells (orange arrow). The yellow square in the inset indicates the area of close-up. Bars: (a, e), 500 μm; (b, c), 200 μm; (d, f), 10 μm.

in the second biological replicate (data not shown). The abundance of *LePFP* β subunit PCR product was lower than that in tomato and seemed to be unrelated to the distance from the host (compare 1 and 5 cm to 15 and 20 cm in Fig. 4). The *LePFP* β subunit transcript became undetectable beyond 30 cm. To ensure the cDNA integrity, the dodder *PFP* (*CpPFP*) β subunit was used as a positive control. These results suggest that the host *PFP* β subunit mRNA is transferred from host to dodder and remains stable in the dodder phloem stream for a long distance.

Discussion

The work presented here demonstrates that translocation of mRNA molecules from host to dodder parasite occurs via parenchyma cells and phloem and that the mRNA molecules are stable for a long distance in dodder. This translocation was observed from two host plant species into the parasitic plant dodder. We detected four tomato mRNAs in dodder grown on tomato (Fig. 2). Interestingly, all transcripts used in this study appeared to move, including the tomato RuBisCo small subunit, which in the distantly related species *Cucurbita maxima* was shown not to be present in the phloem sap (Ruiz-Medrano *et al.*, 1999). Two possibilities that may explain this result are that *LeRbc*S may be present in the phloem sap of tomato or that this particular mRNA species may be translocated into dodder via plasmodesmata connections from host parenchyma. To ascertain if the potential for trans-specific mRNA movement was a consequence of the phylogenetic relatedness of tomato and dodder, which are both in the Asteridae, a second more distantly related host was chosen. Alfalfa belongs to the Eurosids, which diverged from the Euasterids over 100 Myr ago (Yau-Wen *et al.*, 1999). Despite the evolutionary distance between host and parasite, the mRNA for the alfalfa PFP α subunit is detectable in the dodder grown on alfalfa (Fig. 2). Recently, it was also shown that mRNAs are

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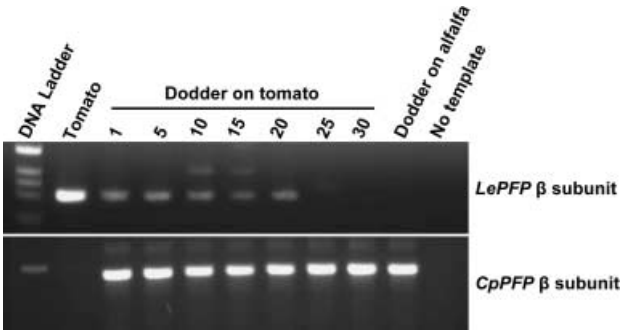

Fig. 4 Reverse transcriptase–polymerase chain reaction (RT-PCR) amplification of tomato (*Solanum lycopersicum*) transcripts along the dodder (*Cuscuta pentagona*) stem away from the host. Dodder cDNA was made from segments at 5-cm intervals up to 64 cm. The upper panel shows the presence of *PYROPHOSPHATE* (*PPi*)*-DEPENDENT PHOSPHOFRUCTOKINASE* (*LePFP*) β transcript from 1 to 20 cm in the first 30 cm. Amplification from tomato was used as a positive control whereas *CpPFP* β transcript was used to ensure cDNA integrity (lower panel). Two independent biological replicates were employed for this experiment. The negative control was a PCR reaction without a template (no template).

translocated from pumpkin to dodder (Roney *et al.*, 2007). Together, these results suggest that cross-species RNA exchange between dodder and host may be a widespread phenomenon.

It was suggested that parasitic plants that rely on obtaining host phloem may not be able to selectively import phloem contents (Birschwilks *et al.*, 2006). Indeed, all four gene transcripts that were analyzed here were transported to dodder. However, in a recent study it was shown that not all phloem mRNA molecules are detected in dodder cDNA (Roney *et al.*, 2007). However, the possibility of technical limitations precluding detection of small amounts of these transcripts was not ruled out (Roney *et al.*, 2007). Selective protein transport was evident in transgenic Arabidopsis expressing a GFP-ubiquitin fusion in the phloem (Birschwilks *et al.*, 2007), yet the fusion protein was not shown to be mobile. We speculate that mRNA molecules move from host to dodder in a nonselective manner.

Direct phloem connections are known to form in several host–parasitic plant interactions. For example, *Orobanche* forms direct plasmodesmatal connections with its hosts which develop into sieve pores connecting the vascular systems of the two organisms (Kuijt & Toth, 1976; Kollmann & Dorr, 1994; Perez-De-Luque *et al.*, 2005). In dodder, it has been previously suggested that the parenchyma cells in the haustorium differentiate into phloem elements in order to form a sieve tube connection with the host (Truscott, 1958). In addition, Truscott (1958) suggested that the phloem of the central stele is separated into small groups of sieve tube elements and companion cells. He showed that the sieve tube connection between host and parasite is probably not direct, but rather via specialized parenchyma cells (Truscott, 1958). We showed the localization of tomato *PFP* α subunit in the dodder parenchyma cells as well as in the phloem (Fig. 3). Therefore, these results imply that mRNA moves from the host to the parasite via symplastic continuity through parenchyma cells to the dodder phloem. Nevertheless, these findings do not exclude the possibility that mRNA molecules also move through an unobserved direct phloem–phloem connection.

The host transcripts, once in dodder, appear to be stable for long distances (Fig. 4). In other studies, many different mRNAs and proteins have been shown to travel long distances within the phloem of several dicot species (Hibberd & Jeschke, 2001; Aoki *et al.*, 2005; Haywood *et al.*, 2005). In certain instances, long-distance mRNA movement can convey important developmental signals and transmit mutant phenotypes across graft junctions (Kim *et al.*, 2001). Phloem mobile mRNAs have been shown to be specifically localized, such as the *Mouse ears* mutant transcript in tomato (Kim *et al.*, 2001) and *Cucurbita maxima NAC family* (*CmNACP*) in pumpkin–cucumber graft experiments (Ruiz-Medrano *et al.*, 1999), and presumably translated into protein at the destination. Our results show that tomato transcripts are present up to the 20–30-cm range, while in more remote portions we could not detect the transcript. Although *LePFP* β was not detectable beyond these distances other more stable transcripts may be detectable at greater distances. We assume that the processes of mRNA degradation and synthesis in the dodder result in a decreasing proportion of host mRNA at greater distances. The presence of host transcripts in dodder at significant physiological distances raises the possibility that host mRNAs may be undergoing localization and translation within dodder. Thus, it would be interesting to investigate *in situ* host transcripts undergoing post-translocation localization by using dodder as a model parasite. In addition, further investigation into this phenomenon should focus on verifying translation of translocated transcripts in the dodder system.

The result of cross-species macromolecular trafficking of proteins and mRNAs may allow developmental coordination between a parasite and its host. This phenomenon was observed by Fratianne (1965), where the flowering time of dodder was synchronous with that of its host. Dodder grown on long-day and short-day plants only flowered when the host was induced to flower. Dodder grown on soybean (*Glycine max*) could be triggered to flower by moving the host to an inductive photoperiod (Fratianne, 1965). Coupled with the recent work showing that the signal that induces flowering is in fact a phloem mobile protein (Corbesier *et al.*, 2007), this raises the possibility that dodder may be utilizing host phloem messages to regulate its developmental processes. Furthermore, we observe that host transcript can move significant distances once it has entered the parasite vascular stream (Fig. 4), raising the possibility that host mRNA molecules could act as long-distance developmental signals in the parasite.

As a consequence of cross-species RNA movement, a mechanism may exist that explains the phenomenon of horizontal gene transfer between parasitic plants and their hosts. Horizontal gene transfer has been demonstrated in flowering plants (Bergthorsson *et al.*, 2003), and recently has also been shown to

occur from dodder to its host (Mower *et al.*, 2004). Movement of phloem contents from a host into dodder is a well-documented phenomenon, but movement of solutes has also been observed in the reverse direction. Carbon^{14}-labeled sucrose applied to a plant infected with dodder can be detected in a second host connected by the same dodder strand (Birschwilks *et al.*, 2006). Although our results did not show the presence of dodder transcript in either host, we did not investigate situations where water stress in the host could lead to a reversal in the flow of solutes from the parasite into the host. This could help explain the enigma of parasite genes being transferred into hosts.

It has occurred to us that the long-distance passage of RNA into the parasitic organism symplasm, in a nonspecific manner, provides a potential opportunity to use gene silencing technologies to generate a mechanism of resistance in crop plants. Further studies are warranted to examine the movement of small interfering RNAs (siRNAs) across host–parasite junctions and the ability of these siRNAs to interfere with vital processes in the parasite.

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References

Aoki K, Suzui N, Fujimaki S, Dohmae N, Yonekura-Sakakibara K, Fujiwara T, Hayashi H, Yamaya T, Sakakibara H. 2005. Destination-selective long-distance movement of phloem proteins. *Plant Cell* **17**: 1801–1814.

Barkman T, McNeal J, Lim S-H, Coat G, Croom H, Young N, dePamphilis C. 2007. Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evolutionary Biology* **7**: 248.

Bergthorsson U, Adams KL, Thomason B, Palmer JD. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature* **424**: 197–201.

Bharathan G, Goliber TE, Moore C, Kessler S, Pham T, Sinha NR. 2002. Homologies in leaf form inferred from knoxi gene expression during development. *Science* **296**: 1858–1860.

Birschwilks M, Haupt S, Hofius D, Neumann S. 2006. Transfer of phloem-mobile substances from the host plants to the holoparasite *Cuscuta* sp. *Journal of Experimental Botany* **57**: 911–921.

Birschwilks M, Sauer N, Scheel D, Neumann S. 2007. *Arabidopsis thaliana* is a susceptible host plant for the holoparasite *Cuscuta* spec. *Planta* **226**: 1231–1241.

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C *et al.* 2007. Ft protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* **316**: 1030–1033.

Dawson JH, Musselman LJ, Wolswinkel P, Dorr I. 1994. Biology and control of *Cuscuta*. *Reviews of Weed Science* **6**: 265–317.

Dorr I. 1996. New results on interspecific bridges between parasites and their host. In: Moreno MT, Cubero JI, Berber D, Joel D, Musselman LJ, Parker C, eds. *Advances in parasitic plant research*. Cordoba, Spain: Junta de Andalucia, 196–201.

Dorr I, Kollmann R. 1995. Symplastic sieve element continuity between *Orobanche* and its host. *Botanica Acta* **108**: 47–55.

Fratianne GD. 1965. The interrelationship between the flowering of dodder and the flowering of some long and short day plants. *American Journal of Botany* **52**: 556–562.

Haupt S, Oparka KJ, Sauer N, Neumann S. 2001. Macromolecular trafficking between *Nicotiana tabacum* and the holoparasite *Cuscuta reflexa*. *Journal of Experimental Botany* **52**: 173–177.

Haywood V, Yu T-S, Huang N-C, Lucas WJ. 2005. Phloem long-distance trafficking of gibberellic acid-insensitive RNA regulates leaf development. *The Plant Journal* **42**: 49–68.

Hibberd JM, Bungard RA, Press MC, Jeschke WD, Scholes JD, Quick WP. 1998. Localization of photosynthetic metabolism in the parasitic angiosperm *Cuscuta reflexa*. *Planta* **205**: 506–513.

Hibberd JM, Jeschke DW. 2001. Solute flux into parasitic plants. *Journal of Experimental Botany* **52**: 2043–2049.

Hiosford MR. 1967. Transmission of plant viruses by dodder. *The Botanical Review* **33**: 387–406.

Jeschke WD, Räth N, Bäumel P, Czygan F-C, Proksch P. 1994. Modelling the flow and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L.: I. Methods for estimating net flows. *Journal of Experimental Botany* **45**: 791–800.

Kim M, Canio W, Kessler S, Sinha N. 2001. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. *Science* **293**: 287–289.

Kollmann R, Dorr I. 1994. Symplastic sieve element continuity between *Orobache* and its host. *Botanica Acta* **108**: 47–55.

Kuijt J, Toth R. 1976. Ultrastructure of angiosperm haustoria – a review. *Annals of Botany* **40**: 1121–1130.

Lanini WT, Kogan M. 2005. Biology and management of *Cuscuta* in crops. *Ciencia e Investigación Agraria* **32**: 165–179.

Long JA, Moan EI, Medford JI, Barton MK. 1996. A member of the knotted class of homeodomain proteins encoded by the STM gene of Arabidopsis. *Nature* **379**: 66–69.

Malik CP, Singh MB. 1979. Physiological and biochemical aspects of parasitism in *Cuscuta*: a review. *Annual Review of Plant Sciences* **1979**: 67–112.

Moreno MT, Cubero JI, Berber D, Joel D, Musselman LJ, Parker C. 1997. *Advances in parasitic plant research*. Cordoba, Spain: Junta de Andalucia.

Mower JP, Stefanovic S, Young GJ, Palmer JD. 2004. Plant genetics gene transfer from parasitic to host plants. *Nature* **432**: 165–166.

Parker C, Richard CR. 1993. *Parasitic weeds of the world: biology and control.* Wallingford, UK: CAB International.

Perez-De-Luque A, Rubiales D, Cubero JI, Press MC, Scholes J, Yoneyama K, Takeuchi Y, Plakhine D, Joel DM. 2005. Interaction between *Orobanche crenata* and its host legumes: unsuccessful haustorial penetration and necrosis of the developing parasite. *Annals of Botany* **95**: 935–942.

Roney JK, Khatibi PA, Westwood JH. 2007. Cross-species translocation of mRNA from host plants into the parasitic plant dodder. *Plant Physiology* **143**: 1037–1043.

Ruiz-Medrano R, Xoconostle-Cazares B, Lucas WJ. 1999. Phloem long-distance transport of CmNACP mRNA: implications for supracellular regulation in plants. *Development (Cambridge)* **126**: 4405–4419.

Truscott FH. 1958. On the regeneration of new shoots from isolated dodder haustoria. *American Journal of Botany* **45**: 169–177.

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Tsivion Y. 1978. Loading of assimilates and some sugars into the translocation system of *Cuscuta*. *Australian Journal of Plant Physiology* **5**: 851–857.

Vaughn KC. 2003. Dodder hyphae invade the host: a structural and immunocytochemical characterization. *Protoplasma* **220**: 189–200.

Yau-Wen Y, Kun-Nan L, Pon-Yean T, Wen-Hsiung L. 1999. Rates of nucleotide substitution in angiosperm mitochondrial DNA sequences and dates of divergence between *Brassica* and other angiosperm lineages. *Journal of Molecular Evolution* **48**: 597–604.



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