


ARTICLE ADDENDUM



Differentiation of vascular elements in haustoria of *Cuscuta japonica*

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ABSTRACT

Parasitic plants establish vascular-conducting cells in an intrusive organ called haustorium. In haustoria of a stem parasitic plant, *Cuscuta japonica*, the presence of cells expressing cell-type-specific genes of phloem companion cell, phloem sieve element, procambial cell and xylem vessel has recently been demonstrated. Differentiation of these vascular cells is regulated in a manner similar to that in conventional vascular tissues. However, the initiation of procambial cells occurs concomitantly with the differentiation of vascular-conducting cells. The differentiation process of phloem also differed from that of conventional vascular tissues because enucleation of sieve elements appeared to be impeded. These results collectively imply that the vascular differentiation process in haustoria of parasitic plants may be different from that in conventional vascular tissues.

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Parasitic plants establish vascular-conducting cells in an intrusive organ called haustorium. A serial array of haustorial vascular-conducting cells serves as a bridge between the vascular systems of host and parasite plants and facilitates the transport of water and nutrients from host to parasite plants. Experiments using various tracer dyes demonstrated that haustorial vascular-conducting cells function in the transport of substances from the host to the parasite.¹⁻³ However, it remains unclear whether the differentiation of vascular-conducting cells in haustoria occur in the same manner as in conventional vascular tissues.⁴⁻⁷

We have recently revealed that cell type-specific genes are expressed in haustoria of a stem parasitic dodder, *Cuscuta japonica* (*Cj*).⁸ Expression of *CjAPL*, *CjSEOR1*, *CjWOX4* and *CjTED7* was confirmed in *Cj* haustoria, demonstrating that phloem companion cells,⁹ phloem sieve elements,¹⁰ procambial cells¹¹ and developing xylem vessels¹² are present in haustoria, respectively. In addition to these cell type-specific genes, we confirmed the expression of genes that regulate vascular cell differentiation,¹³ including *CjCLE41*, *CjGSK3* and *CjBES1* in haustoria.⁸ The expression level of *CjCLE41*, which encodes a peptide called the tracheary element differentiation inhibitory factor (TDIF) that maintains the activity of the stem cell,¹¹ started to increase at 72 hour after attachment (h.a.a.), which is just before the differentiation of xylem vessels in haustoria, and it showed the maximum level at 96 h.a.a. when xylem vessels started to differentiate. Expression of *CjGSK3* continuously decreased, while that of *CjBES1* continuously increased from 72 to 120 h.a.a., which coincided with the formation of continuous xylem vessel. These results suggest that the TDIF-TDR-GSK3 signaling pathway controls the differentiation of xylem

in haustoria in a manner similar to that in roots and stems. In situ hybridization demonstrated that the expression of *CjWOX4* and *CjCLE41* was localized in the adjacent cells, but was not overlapped with the haustorial xylem vessel, suggesting that distinct phloem/procambial- and xylem domains were established in haustoria.⁸

In haustoria, the expression of *CjWOX4* and *CjCLE41*, both of which are involved in the maintenance of procambial cells, and *CjAPL*, *CjSEOR1* and *CjTED7*, which are cell type-specific genes, showed a remarkable increase from 72 to 96 h.a.a.⁸ These results suggest that differentiation of procambial- and vascular-conducting cells started concomitantly, and the latter continued until the later stages of haustorial development (Fig. 1). However, it remains unclear how procambial cells are initiated in this differentiation scheme (Fig. 1). Haustorial cells of *Cj* are initiated in the cortex of stem by forming a meristem-like region called prehaustorium. The expression level of *CjWOX4* was much lower in prehaustoria at 48 h.a.a. than in haustoria at 72 h.a.a.⁸ We did not successfully identify cells expressing *CjWOX4* using in situ hybridization in prehaustoria at 48 h.a.a. At 72 h.a.a., cells expressing *CjWOX4* were localized in the central part of the haustorium, suggesting that specification of procambial cells was already completed before the onset of xylem vessel formation.⁸ These results suggest two possible hypotheses. Procambial cells might be initiated in haustoria (not in prehaustoria) when they were elongated (Fig. 1, right). Alternatively, an identity of procambial cells might have been established in prehaustoria (Fig. 1, left) and expression level of *CjWOX4* increased during the elongation of haustorial cells because elongating cells increase their ploidy, as observed in dark-grown

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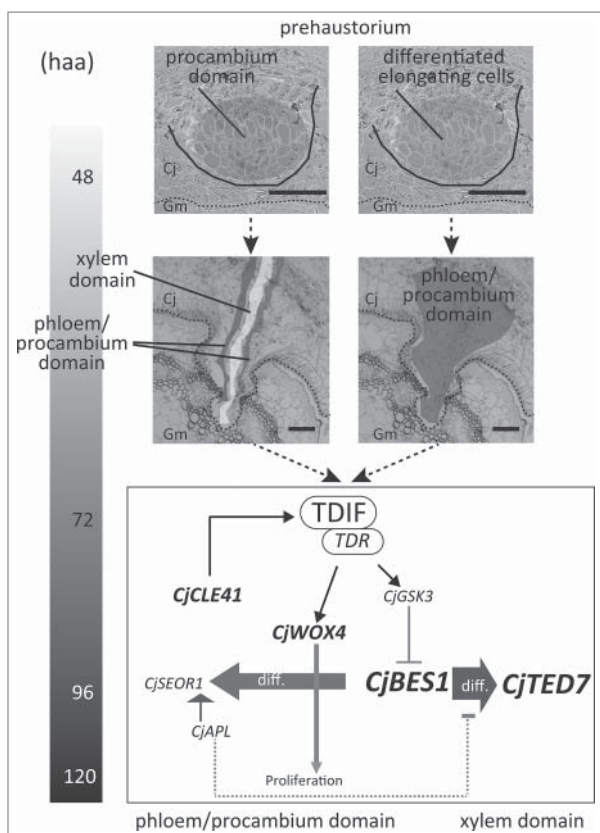


Figure 1. Differentiation of vascular-conducting cells in haustoria of *Cuscuta japonica*. Upper: In the prehaustorium, cells started elongating. *CjWOX4*, a procambium-specific gene, was not detected either by RT-PCR or in situ hybridization.⁸ Thus, it is unclear whether procambial cells are established (grey, left) or not (right) in the prehaustorium. Middle: between 48 and 72 h.a.a., before the onset of xylem vessel formation (white, left), procambial cells expressing *CjWOX4* (grey, left) were established. This does not exclude the possibility that phloem and xylem has been differentiated. Lower: after 72 h.a.a., haustorial procambial cells differentiated into distinct phloem/procambial- and xylem domains. Solid line indicates the boundary of the prehaustorium. Dotted line indicates the boundary of the parasite, *Cuscuta japonica* (Cj) and the host, *Glycine max* (Gm). Scale bar, 50 μ m.

hypocotyls.¹⁴ To test these hypotheses, a methodology enabling higher-sensitivity detection of gene expression, such as transformation using promoter-reporter constructs, needs to be developed for *Cuscuta* spp.

Differentiation of phloem-conducting cells in haustoria also appears to be different from the conventional phloem. Although phloem-conducting cells are arranged in a linear conductive array, some of the cells contain nuclei,⁸ suggesting that they are not mature sieve elements (SE). The presence of nucleated SEs has been reported in several parasitic plants including *Cuscuta gronovii*,¹⁵ *Alectra vogelii*,¹⁶ and *Phelipanche ramosa*.¹⁶ Recently, the presence of nucleated phloem-conducting cells has also been reported in haustoria of *Phelipanche aegyptiaca*.¹⁷ In haustoria of *P. aegyptiaca*, a homolog of NAC-DOMAIN CONTAINING TRANSCRIPTION FACTOR 45/86 (*NAC45/86*), *PaNAC45*, and homologs of *NAC45/86-DEPENDENT EXONUCLEASE-DOMAIN PROTEIN 1* (*NEN1*) and *NEN4*, *PaNEN1* and *PaNEN4*, were expressed in higher levels than in the protrusion organs of the tubercle in which enucleated SEs are differentiated.¹⁷ *NAC45/86* encodes a transcription factor protein that regulates the downstream processes of SE maturation, and *NEN1* and

NEN4 encode exonucleases responsible for the degradation of nuclei in SE.¹⁸ In *Cj*, the expression of *CjNEN4* in haustorium was confirmed and it increased from 72 to 96 h.a.a. (Fig. 2A). The contradiction between the retention of nuclei and expression of an exonuclease gene implied a mechanism repressing the nuclear degradation. Establishment of SE is controlled by the OCTOPUS-BRASSINOSTEROID INSENSITIVE 2-BRI1-EMS-SUPPRESSOR 1 (*OPS-BIN2-BES1*) regulatory pathway that controls SE differentiation and the ALTERED PHLOEM DEVELOPMENT (*APL-NAC45/86-NEN*) regulatory pathway that controls SE enucleation (Fig. 2B).¹⁹ Nucleated SEs are frequently observed in the *ops* loss-of-function mutant of *Arabidopsis*,^{20,21} which implies that the expression of *OPS* may be lower in haustorial phloem-conducting cells than in the conventional phloem. The occurrence of a crosstalk between *OPS-BIN2-BES1* and *APL-NAC45/86-NEN* regulatory pathways needs to be clarified (Fig. 2B).

In conclusion, the presence of cells that have attributes of vascular elements was confirmed in *Cj* haustoria. Differentiation of these elements was regulated by *TDIF-TDR-WOX4*- and *TDIF-TDR-GSK3* regulatory pathways in a manner similar to that in conventional vascular tissues. However, the initiation of procambial cells may differ. The differentiation process of SE also appears to differ from that in conventional vascular tissues because enucleation of SE appears to be impeded. This implies a possible crosstalk between the *OPS-BIN2-BES1* and *APL-NAC45/86-NEN* regulatory pathways.

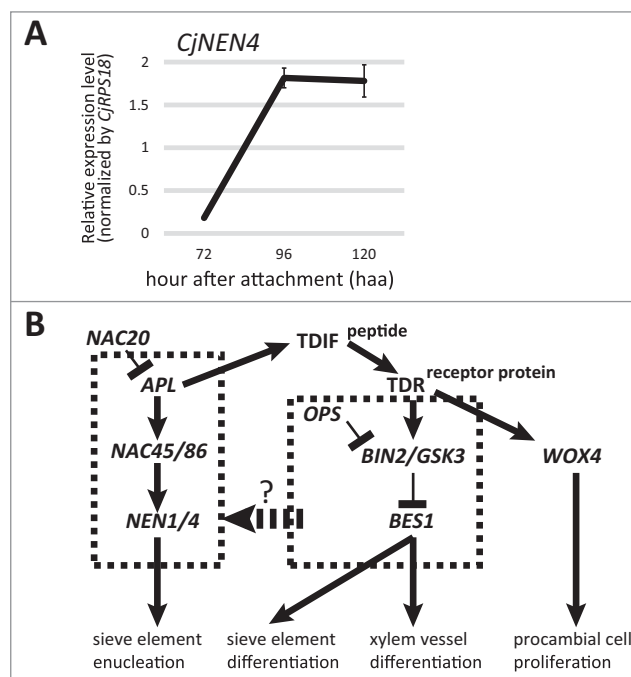


Figure 2. Regulation of phloem-conducting cell differentiation in haustoria of *Cuscuta japonica*. (A) Expression profile of *CjNEN4* in haustoria. Expression level increased from 72 to 96 h.a.a., which is inconsistent with the retention of nuclei. Expression levels were normalized by that of *C. japonica* ribosome protein *S18* gene (*CjRPS18*). Mean and standard error of three biological replicates are indicated. (B) Hypothesis for the regulation of differentiation and enucleation of phloem-conducting cells. Main hypothesis is that *OPS-BIN2-BES1* regulatory pathway may promote the enucleation by controlling *APL-NAC45/86-NEN* regulatory pathway, although the point of action has not been identified (dotted arrow). In *Cj* haustoria, expression of *OPS*, *BIN2*, or *BES1* may be lower than conventional vascular tissues, which results in retention of nuclei in phloem-conducting cells.

Abbreviations

APL	ALTERED PHLOEM DEVELOPMENT
BES	BRI1-EMS-SUPPRESSOR
BIN	BRASSINOSTEROID-INSENSITIVE
Cj	<i>Cuscuta japonica</i>
CLE	CLAVATA3/EMBRYO SURROUNDING REGION-RELATED
GSK	glycogen synthase kinase
haa	hours after attachment
NEN	NAC45/86-DEPENDENT EXONUCLEASE-DOMAIN PROTEIN
OPS	OCTOPUS
SE	sieve element
SEOR	SIEVE ELEMENT OCCLUSION-RELATED
TDIF	tracheary element differentiation inhibitory factor
TDR	TDIF-receptor
TED	TRACHEARY ELEMENT DIFFERENTIATION-RELATED
WOX	WUSCHEL-RELATED HOMEODOMAIN

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