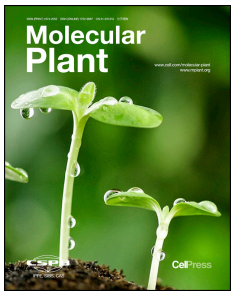
Journal Pre-proof



Extensive inter-plant protein transfer between *Cuscuta* parasites and their host plants

Nian Liu, Guojing Shen, Yuxing Xu, Hui Liu, Jingxiong Zhang, Shalan Li, Jing Li, Cuiping Zhang, Jinfeng Qi, Lei Wang, Jianqiang Wu

PII: S1674-2052(19)30398-3
DOI: https://doi.org/10.1016/j.molp.2019.12.002
Reference: MOLP 861

To appear in: *MOLECULAR PLANT*
Accepted Date: 27 November 2019

Please cite this article as: **Liu N., Shen G., Xu Y., Liu H., Zhang J., Li S., Li J., Zhang C., Qi J., Wang L., and Wu J.** (2020). Extensive inter-plant protein transfer between *Cuscuta* parasites and their host plants. Mol. Plant. doi: https://doi.org/10.1016/j.molp.2019.12.002.

Extensive inter-plant protein transfer between *Cuscuta* parasites and their host plants

Nian Liu[1,2], Guojing Shen[1], Yuxing Xu[1,2], Hui Liu[1], Jingxiong Zhang[1,2], Shalan Li[1,2], Jing Li[1], Cuiping Zhang[1,2], Jinfeng Qi[1], Lei Wang[1], and Jianqiang Wu[1]*

[1]Department of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[2]University of the Chinese Academy of Sciences, Beijing 100049, China

*Correspondence to: wujianqiang@mail.kib.ac.cn.

Running title: Protein transfer between *Cuscuta* and its hosts

Short Summary: Hundreds to more than one thousand proteins were found to be transferred between *Cuscuta* parasites and their hosts. Furthermore, large-scale protein exchange occurred between different hosts through *Cuscuta* connections. The translocated proteins may retain their activity and play important roles in the interactions between *Cuscuta* and hosts and among *Cuscuta*-connected hosts.

ABSTRACT

Cuscuta species (dodders) are holoparasites that totally rely on host plants to survive. Although various mobile proteins have been identified to travel within a plant, whether and to what extent protein transfer between *Cuscuta* and host plants remain unclear. We found that hundreds to more than 1500 proteins were transferred between *Cuscuta* and the host plants Arabidopsis and soybean, and hundreds of inter-plant mobile proteins were even detected in the seeds of *Cuscuta* and the host soybean. Different hosts bridge-connected by dodder were also found to exchange hundreds of proteins. Quantitatively, the mobile proteins represent a few to more than 10% of the proteomes of the foreign plants. Using Arabidopsis plants expressing different reporter proteins, we show that these reporter proteins could travel between plants, and importantly, retained their activity in the foreign plants. Comparison between the inter-plant mobile proteins and mRNAs indicated that the majority of the mobile proteins were not *de novo* synthesized from the translocated mRNAs, but *bona fide* mobile proteins. We propose that large-scale inter-plant protein translocation may play an important role in the interactions between host plants and dodder and even among the dodder bridge connected hosts.

Keyword: dodder, *Cuscuta*, host plants, protein transfer, plant-plant interaction

INTRODUCTION

Approximately 1% of all flowering plants, 4000-5000 species in 12 or 13 lineages, have evolved the parasitic lifestyle (Westwood et al., 2010). *Cuscuta* species (Convolvulaceae), commonly known as dodders, are root- and leafless plant parasites, which do not or barely photosynthesize. Like other plant parasites, dodder uses a specific organ named haustorium to penetrate into hosts and extract water and nutrients (Clarke et al., 2019). A dodder parasite grows many stems, which twist around the host, and along the dodder stems, many haustoria are developed. Haustorial connections very likely allow flow or even exchange of various molecules between dodder and hosts. It has been long known that viruses (Birschwilks et al., 2006; Hosford, 1967) and phytoplasmas (Kaminska and Korbin, 1999) can be transmitted between dodder and hosts. RNA-seq analysis also indicated trafficking of thousands of mRNA species between hosts and dodder (Kim et al., 2014). miRNAs from dodder can migrate into hosts and induce silencing of defense-related genes, facilitating the establishment of parasitism (Shahid et al., 2018). Secondary metabolites can be translocated from hosts into dodder and even protect dodder from insect feeding (Smith et al., 2016). Insect feeding-induced systemic signals are also transferred from dodder to host plants, as well as among different hosts through dodder bridge connections, inducing host defense against insects (Hettenhausen et al., 2017; Zhuang et al., 2018).

Within a plant, various small molecules, mRNAs, small RNAs, and proteins traffic between different cells, tissues, and organs (Batailler et al., 2012; Du et al., 2015; Hu et al., 2016; Liu and Chen, 2018; Lopez-Cobollo et al., 2016; Lucas et al., 2013; Ostendorp et al., 2017; Paultre et al., 2016; Thieme et al., 2015). Although the biological significance of the movement of the mobile mRNAs and proteins remains largely elusive, some of these mRNAs and proteins likely play important roles in coordinating the growth and development of different plant parts and plant adaptation to stresses (Kim et al., 2001; Lough and Lucas, 2006; Winter and Kragler, 2018). For example, day length is perceived by leaves and activates the biosynthesis of a mobile protein signal, named flowering locus T (FT), which moves through the phloem to the shoot apex to induce flowering (Turck et al., 2008). In the tomato *mouse*

ears (*me*) mutant, the *pyrophosphate-dependent phosphofructokinase* (*PFP*) is fused with *LeT6*, which is a tomato knotted-1-like homeobox gene, and this fusion transcript is able to move from the *me* stock to wild-type (WT) tomato scion and changes the morphology of WT scion (Kim et al., 2001).

A few lines of evidence have indicated that proteins can be translocated from hosts to dodder. Green fluorescence protein (GFP) expressed in tobacco (*Nicotiana tabacum*) or Arabidopsis (*Arabidopsis thaliana*) was found to be transferred into dodder (Birschwilks et al., 2007; Haupt et al., 2001). Soybean (*Glycine max*) transformed with *phosphinothricin acetyl transferase* (*PAT*) is resistant to the herbicide glufosinate, and dodder parasitizing on these soybean plants exhibited increased tolerance to glufosinate than did the dodder grown on the WT soybean, and PCR analysis ruled out the possibility of the transfer of *PAT* transcripts from transgenic soybean to dodder (Jiang et al., 2013). However, whether and to what extent proteins are transferred between dodder and hosts are mostly unclear.

Recently, the genomes of *C. australis* and *C. campestris* were sequenced and analyzed (Sun et al., 2018; Vogel et al., 2018). The wide host range of dodder also allows distantly related host plants to be selected, which in turn, facilitates proteomic discrimination of proteins from different organisms based on the large sequence divergences among the homologous proteins in dodder and hosts. With proteomic analyses, we found hundreds to more than one thousand proteins to be translocated between the stems of dodder and those of Arabidopsis or soybean. Large numbers of these inter-plant mobile proteins were also found in the seeds of dodder and soybean plants. Moreover, when dodder bridge connected Arabidopsis and soybean plants, the exchange of hundreds of proteins between these two hosts occurred. The quantities of protein transferred were estimated to represent a few to more than 10% of the proteomes of these plants and their seeds. Using transgenic host plants expressing different reporter proteins, we showed that these reporter proteins retained their activity after inter-plant movement. Comparison between the inter-plant mobile proteins and mRNAs indicated that the majority of the mobile proteins were translocated from foreign plants, but not *de novo*

synthesized from the mobile mRNAs. This extensive inter-plant protein trafficking mediated by parasitic dodders may influence the recipient plants' physiology.

RESULTS

Protein transfer between host and dodder stems

To examine the protein transfer between dodder and host plants, we infested Arabidopsis and soybean plants with dodder (*C. australis*), and when the parasites were well-established, Arabidopsis stems, soybean stems, and two segments of dodder stems that were proximal and distal to the host stems were collected for label-free peptide quantitation proteome analyses. Three biological replicates were used for each group of samples, and for each group only peptides that appeared in at least two out of three replicates were considered to be positively identified. The sequences of positively identified peptides were mapped to the dodder and host (Arabidopsis or soybean) genome, if any did not match either genome (because of sequencing errors and posttranslational modifications, etc.), they were filtered out; subsequently, 1) peptides which matched to both genomes and only matched to native genome were considered to be from native proteins; 2) peptides that only matched the foreign plant genome but not the native plant genome were considered to be mobile proteins.

Six hundred-nineteen and 615 Arabidopsis proteins and 1535 and 1547 soybean proteins were identified in dodder proximal and distal stem segments, respectively, which accounted for 27.0% (734/2714) and 37.9% (1807/4765) of the total Arabidopsis and soybean protein species identified in the respective host stems (Figure 1A, 1B, and Supplemental Data 1). Moreover, 1027 and 1674 dodder proteins were detected in Arabidopsis and soybean stems, respectively, and these were 17.4 (1027/5914) and 28.3% (1674/5914) of the total dodder protein species detected in dodder stems (Figure 1A, 1B, and Supplemental Data 1). Although the host plant-produced proteins were mostly distributed throughout dodder proximal and distal stem segments, some proteins were only detected in one or the other of these two dodder stem segments (Figure 1C). We speculate that proteins specifically detected

in proximal parts may have high turnover rates, making them undetected in distal segments; in contrast, some rapidly-transported host proteins might accumulate in dodder distal segments and not be detected in the proximal parts.

Notably, even though transcription factors (TFs) usually have low protein abundance and thus are hard to be detected in proteome analyses, 6 Arabidopsis and 8 soybean TFs were found to be translocated from hosts to dodder (Supplemental Table 1). Four and 9 dodder TFs were transferred into Arabidopsis and soybean, respectively (Supplemental Table 1). In addition to TFs, some potential pathogen- (such as R proteins) and insect-resistant (such as P450s) proteins were translocated into the foreign plants and may confer resistance to biotic stresses (Supplemental Table 2): two R proteins and one P450 from Arabidopsis and two R proteins and two P450s from soybean were transferred into dodder stems, and from dodder, one R protein and one P450 were translocated into Arabidopsis; and one R protein and five P450s were translocated into soybean stems.

Previously, more than 1100 proteins had been identified in cucumber and watermelon phloem sap samples (Hu et al., 2016). Thus, combining our inter-plant mobile proteins and those found in Cucurbitaceae, we next asked which mobile proteins are specific and which ones are common in different plant families. Taking advantage of orthology and Venn diagram analysis, it was found that that 54 protein species are likely to be conserved in long-distance trafficking (Figure 1D), and gene Ontology (GO) analysis indicated that these 54 conserved protein species are mainly related to adaxial/abaxial pattern specification, cell wall pectin metabolic process, gluconeogenesis, response to glucose, actin filament bundle assembly, and trehalose metabolism in response to stress (Supplemental Data 2). One hundred and forty-eight dodder proteins were found to be specifically dodder-produced mobile proteins (Figure 1D), and they were translocated to both Arabidopsis and soybean, implying that some dodder-specific proteins may be important for dodder parasitism. GO analysis revealed that these 148 dodder proteins are enriched in GTP biosynthetic process, UTP biosynthetic process, nucleoside diphosphate phosphorylation, tricarboxylic acid cycle, and CTP biosynthetic process (Supplemental Data 2).

Previously, it was shown that thousands of mRNAs were translocated between dodder and hosts (Kim et al., 2014). To compare the inter-plant protein and mRNA transfer, the transcriptomes of the same tissues from Arabidopsis-dodder and soybean-dodder systems, which were used for the above proteomic analyses, were sequenced to obtain inter-plant mobile mRNAs, except that the proximal and distal stems were equally mixed before samples were sequenced (three biological replicates for each group), since the mobile host proteins that found in dodder proximal and distal stem segments had little differences and RNA-seq was considered to be much more sensitive than proteomic analysis to detect the foreign mRNAs. In the Arabidopsis-dodder system, only 0.06% and 0.28% reads were originated from foreign plants in the dodder and Arabidopsis transcriptomes, respectively (Supplemental Figure 1A and Supplemental Data 3, the percentages are the reads number only mapped to the foreign genome to the total reads number that mapped to the foreign and native genome); the analysis of distribution of count numbers (CNs) of foreign and native mRNAs also indicated that the abundance of individual mobile mRNAs was much less than was that of the native ones (Supplemental Figure 1A). In contrast, in Arabidopsis stems the ratio between the mass spectrometry (MS) total precursor intensities (TPIs) of dodder proteins and those of all proteins was 16.68%. In dodder stems (including both proximal and distal segments), 4.30 to 4.53% of the TPIs of dodder stem proteins were contributed by the Arabidopsis mobile proteins (Figure 1E). Generally, the estimated relative protein abundance (ERPA) of most foreign proteins was lower than the native proteins, but compared with the relative abundance of foreign mRNAs, the individual mobile proteins were much greater in their ERPA (Figure 1E and Supplemental Figure 1A). Similarly, in soybean-dodder system, dodder transcriptome contained 1.22% of soybean reads and soybean transcriptome contained only 0.19% of dodder reads (Supplemental Figure 1A and Supplemental Data 3); however, 13.70% of the TPIs in soybean proteome and 10.95 to 11.17% of TPIs in dodder proteome were from dodder and soybean proteins, respectively (Figure 1E). Again, the translocated proteins' relative abundance was generally much higher than that of mRNAs (Figure 1E and Supplemental Figure 1A). Furthermore, among the 734 Arabidopsis proteins and 172 Arabidopsis mRNAs that were found in dodder stems, only 14 were co-transferred

(Supplemental Figure 1B). Similarly, comparison of the 1027 dodder proteins and 1417 dodder mRNAs identified in Arabidopsis stems revealed that transcripts and proteins from 381 dodder loci were co-transferred (Supplemental Figure 1B). In the soybean-dodder system, we found no soybean mRNAs and proteins to be co-transferred to dodder stems, and among all the proteins (1674) and mRNAs (708) transferred from dodder to soybean stems, transcripts and proteins from 259 dodder loci were co-transferred (Supplemental Figure 1C). These data suggest that these inter-species mobile proteins are mostly translocated proteins, but not *de novo* synthesized from the mobile mRNAs in the foreign plants.

Activity of inter-plant transferred reporter proteins

To further evaluate inter-plant protein trafficking and examine whether inter-plant mobile proteins retain their activity after long-distance transfer, Arabidopsis plants expressing five reporter proteins, eGFP (enhanced GFP)-GUS (β-glucuronidase) fusion protein (90 kDa), GUS (68 kDa), LUC (luciferase) (60 kDa), eGFP (27 kDa), and PAT (phosphinothricin acetyl transferase, 22 kDa) (Supplemental Table 3) were prepared, and these plants were infested with dodders. GUS activity was clearly detected in the stems of dodder grown on the Arabidopsis plants expressing eGFP-GUS or GUS (Figure 2A to C and Supplemental Figure 2A). Importantly, eGFP-GUS protein was specifically located in the sieve cells of dodder (Figure 2A), indicating that it was translocated through phloem. Similarly, LUC activity was detected in the proximal and distal parts of dodder stems, whose hosts expressed LUC (Figure 2D and Supplemental Figure 2B). Using Western blotting, eGFP was clearly detected in the proximal and distal dodder segments when they grew on Arabidopsis expressing eGFP (Figure 2E), although eGFP fluorescence was undetected visually, probably due to the dodder autofluorescence and the relatively low levels of transferred eGFP. Furthermore, consistent with what was found in dodder grown on glufosinate-resistant soybean (Jiang et al., 2013), dodder parasitizing Arabidopsis plants which had been transformed with the *PAT* gene (*phosphinothricin acetyl transferase*, which encodes a 22-kDa protein) became tolerant to glufosinate (Supplemental Figure 2C). According to our proteomic data, a jasmonic acid (JA) biosynthetic enzyme AOS (allene oxide synthase) was transferred between dodder and

soybean hosts (Supplemental Data 1). To further confirm the movement of AOS, Arabidopsis *AOS* fused with a *FLAG* tag (58 kDa) was expressed in Arabidopsis under the control of its native promoter. AOS-FLAG was clearly detected in dodder stems when they grew on Arabidopsis expressing AOS-FLAG, while the dodder stems on the WT Arabidopsis had no detectable signals (Figure 2F). We also examined whether the transcripts of these six transgenes could be detected in dodder with PCR, but no PCR products were obtained (Supplemental Figure 2D).

Therefore, eGFP-GUS, GUS, LUC, eGFP, PAT, and AOS proteins were translocated from Arabidopsis into dodder stem, but not translated from the mobile Arabidopsis mRNAs. Importantly, these mobile eGFP-GUS, GUS, LUC, and PAT retained their activity in the foreign plants, although the activity of eGFP and AOS was not detected due to the low level of eGFP and complex biochemical analysis procedure for AOS.

Inter-plant translocation of proteins to soybean and dodder seeds

Next, we asked whether inter-plant mobile proteins can be translocated into seeds, given our findings of extensive protein trafficking in stems. Dodders were grown on soybean plants until both produced seeds, and the seeds were sampled for proteome analyses (three biological replicates were used for each group of samples; dodder makes no seeds when parasitizing Arabidopsis, thus the Arabidopsis-dodder system was excluded from this analysis). In dodder and soybean seeds, we identified a total of 4186 and 4797 proteins, respectively, and among these, 978 soybean proteins and 608 dodder proteins were found to have been delivered into the seeds of dodder and soybean, respectively (Figure 3A and Supplemental Data 1). The TPIs of these translocated proteins were estimated to quantitatively represent 8.84% and 2.23% of dodder and soybean seed proteome, respectively; the distribution of ERPA of the foreign and native proteins indicated that overall the abundance of individual foreign proteins in the seeds were only moderately less than that of the native proteins (Figure 3B). Although little enrichment of GO terms were found for the proteins from soybean to dodder seeds (Supplemental Table 4), GO analysis on the dodder

proteins that travelled to soybean seeds indicated that the enriched GO terms were mainly related to primary metabolism, including glycolytic process, tricarboxylic acid cycle, and gluconeogenesis, and GO terms oxidation-reduction process and response to heat were also enriched (Supplemental Table 4).

The same samples were used for RNA-seq analyses to study whether there is mRNA transfer to the seeds of hosts and dodder. Indeed, foreign mRNAs accumulated in seeds: 860 soybean mRNAs and 91 dodder mRNAs were identified in dodder and soybean seeds, respectively (Supplemental Data 3). Reads from soybean were estimated to be 0.41% of the total mappable reads in dodder seeds, and 1.37% of the reads from soybean seeds were from dodder, and most of the foreign mRNAs had very low CNs, compared with the native mRNAs (Figure 3B). Comparison between the identities of 978 proteins and 860 mRNAs translocated from soybean to dodder seeds revealed that only 121 soybean loci's transcripts and proteins were co-transferred (Figure 3C). Similarly, among the 608 proteins and 91 mRNAs from dodder, only 17 dodder mRNAs and the corresponding proteins were co-transferred from dodder to soybean seeds (Figure 3C). Similar to what was found in stems, 3 soybean and 3 dodder TFs were found in dodder and soybean seeds respectively (Supplemental Table 1). Two soybean and one dodder P450s were translocated into foreign plant' seeds (Supplemental Table 2).

Next, we intended to demonstrate whether protein translocated from foreign plants to dodder or host seeds could retain their activity and may impact the seed physiology. Dodders were infested on transgenic soybean lines expressing GUS or EPSPS (5-enolpyruvyl-shikimate-3-phosphate synthase, an herbicide glyphosate-resistant protein) and dodders on WT soybean served as comparisons. High GUS activity, which could be detected by GUS staining, was clearly detected in dodder flowers when they grew on soybean expressing GUS (Figure 3D). Even though GUS activity in dodder seeds was only detectable with a fluorescence-based quantitative assay, the GUS activity in the seeds which were harvested from dodders grew on GUS-expressing transgenic soybean was greater than that in the seeds from dodders on the WT soybean (Figure 3E). The seeds of dodders grown on the

EPSPS-expressing soybean plants were germinated under the glyphosate treatment or water (as control condition). Under the control and glyphosate treatment conditions, the germination rates of dodder seeds collected from EPSPS-expressing soybeans were the same, but the germination rate of seeds collected from dodders on the WT soybeans was 30% decreased under glyphosate treatment compared to their germination rate under the control condition (Figure 3F).

Dodder-mediated inter-host protein transfer

In nature, a dodder parasite often simultaneously parasitizes two or multiple host plants, forming dodder-connected plant clusters. Through the dodder bridges, these hosts are indirectly "grafted" together and could exchange macromolecules. We created plant clusters, each containing an Arabidopsis and a soybean as hosts, which were bridge connected by dodders (Figure 4A). Arabidopsis rosette leaves and young soybean cauline leaves were collected for proteome analyses (three biological replicates were used for each group of samples). In Arabidopsis and soybean leaves, in total 4716 and 6193 proteins were respectively identified, and 20.1% and 11.6% (949 and 719) were found to be transferred into the other host (Figure 4A and Supplemental Data 1). Based on the MS-based signal TPIs, the transferred proteins from Arabidopsis to soybean occupied ~ 8.26% of soybean proteome, and ~ 8.80% of Arabidopsis proteome was soybean proteins (Figure 4B). Although most of the mobile proteins showed somewhat lower ERPA than did the native proteins, the ERPA of some foreign proteins was even greater than the averages of native proteins (Figure 4B). These data indicated that by functioning as a conduit, dodder mediates extensive protein trafficking between different host plants.

GO analysis on the proteins transferred from soybean to Arabidopsis resulted in very little GO terms (Supplemental Table 4), but the proteins moved from Arabidopsis to soybean showed enrichment in a number of GO terms, including responses to cadmium, salt stress, and cold (Supplemental Table 4). Notably, among the proteins transferred between

Arabidopsis and soybean, 217 were orthologous, while 657 Arabidopsis and 462 soybean proteins were species-specific (Figure 4C). TFs and insect- and pathogen-resistant proteins were found to be transferred between Arabidopsis and soybean. One Arabidopsis TF was translocated into soybean leaves, and 4 soybean TFs were transferred into Arabidopsis leaves (Supplemental Table 1), and five Arabidopsis and one soybean P450s were transferred into soybean and Arabidopsis leaves, respectively (Supplemental Table 2).

To further examine whether the inter-host transferred proteins could retain their activity, Arabidopsis plants expressing eGFP-GUS or WT Arabidopsis plants were connected with soybeans by dodder bridge connections. Elevated GUS activity was detected in soybean leaves, when the soybeans were bridge-connected with Arabidopsis expressing eGFP-GUS, compared with soybeans connected with WT Arabidopsis (Figure 4D), indicating that eGFP-GUS retained its activity after being transferred to another host. The *AOS* mutant *dde2-2* is male-sterile because of the defect in JA biosynthesis, and *dde2-2* does not make any seeds (von Malek et al., 2002). Five *dde2-2* mutants were individually bridge-connected with five soybeans, which served as AOS suppliers. One out of these five mutants was self-pollinated successfully, and its mutation and homozygosity was confirmed by PCR and sequencing (Figure 4E and Supplemental Figure 3A); furthermore, quantification of the JA content in this *dde2-2* mutant, whose flowering phenotype was rescued, indicated that its JA recovered to the normal level, while the remaining four *dde2-2* had very low JA levels (Supplemental Table 5). In this experiment, only a one in five of the tested *dde2-2* mutants was phenotypically rescued. This was consistent with the results of our proteomic analysis on the leaves of Arabidopsis and soybean, which were bridge connected by dodders: Arabidopsis AOS protein with very large differences of the relative abundance (undetectable in one replicate and the other two showed a 5-fold difference) was detected in soybean leaf samples (Supplemental Figure 3B).

The general properties of mobile proteins

The large numbers of *bona fide* inter-plant mobile proteins (6504 in total from this study) allowed us to investigate whether there are certain general rules that long-distance protein translocation follows. First, Arabidopsis-dodder and soybean-dodder proteomic data were exploited to compare the quantities of mobile proteins in their original and foreign plants. The majority of mobile proteins exhibited strongly decreased MS signals after translocation into the foreign plants (Figure 5A and Supplemental Data 1), indicating their decreased concentrations. This is conceivable, as the translocated proteins cannot be synthesized in the foreign plants and their rates of degradation are greater than the rates of being replenished from the source plants. We used the relative signal intensity ratios (RSIRs; the ratios of the mobile proteins' ERPA in the foreign plants to their ERPA in the native plants) to quantitatively estimate the changes of abundance of the mobile protein after they traveled to the foreign plants. In the stems of Arabidopsis, soybean, and dodder, the RSIRs of 3.9 to 10.2% mobile proteins were between 0.5 and 1, and more than 5% of the mobile proteins' RSIRs exceeded 1 (Supplemental Figure 4). Thus, at least 10% and 18% of the mobile dodder and host proteins remained to have at least 50% of their abundance in their native plants, respectively, after they were translocated to the foreign plants. Remarkably, 0.7 to 22.9% mobile proteins were only detected in foreign plants (Supplemental Figure 4), and these proteins are likely more stable in the foreign plants than in their native plants and probably these proteins' turnover rates are lower than the rates of being translocated.

Next, we sought to study whether the inter-plant mobile proteins possess certain properties that enable them to be mobile. First of all, these inter-plant mobile proteins were found to be more abundant than were the non-mobile proteins in source plants (Figure 5B). Secondly, analyses on the molecular weights of the mobile proteins in phloem saps of cucurbits (Supplemental Figure 5A) from a previous study (Hu et al., 2016) and the mobile proteins identified in our parasitic systems indicated that the mobile proteins mainly ranged from 20 to 70 kDa (Figure 5C to D). In the Arabidopsis-dodder system, using Kolmogorov-Smirnov Test (KS-test) analysis, we found that the size distribution patterns of the dodder native proteins and those trafficked to Arabidopsis (hereafter named MTFP proteins, for "moved to foreign plants") had no difference; however, the distribution patterns of Arabidopsis native

and MTFP proteins were statistically different, and the median of mobile proteins was 5.6 kDa larger than that of the native proteins (Figure 5C). In the soybean-dodder system, KS-test analysis showed that the size distribution patterns of the native and MTFP proteins were also statistically different (Figure 5D): Compared to the native proteins, the medians of MTFP proteins increased 7 kDa and 2.2 kDa for dodder and soybean, respectively (Figure 5D). Thus, the inter-plant mobile proteins tend to be larger than the proteins in the native plants. Remarkably, ~ 20% of the mobile proteins detected in this study were over 70 kDa, among which the largest one reached 611 kDa (At1g67120, midasin). Lastly, the subcellular locations of mobile Arabidopsis, soybean, and dodder proteins were predicted according to their sequence properties (Chou and Shen, 2010). Chloroplast, nucleus, cytoplasm, and mitochondrion were the four principal subcellular locations of these inter-plant mobile proteins (Figure 5E and Supplemental Table 6), and 4.0% of the inter-plant mobile proteins were predicted to be located at endoplasmic reticulum (ER) or Golgi apparatus (Figure 5E and Supplemental Table 6). The mobile proteins identified in the cucurbits (3085) were also analyzed for their subcellular localizations: 21.4, 41.5, 18.8, 5.1, 1.7, and 2.6% proteins were predicted to be in chloroplast, nucleus, cytoplasm, mitochondrion, ER, and Golgi apparatus, respectively (Supplemental Figure 5B and Supplemental Table 6). Hence, generally abundant proteins with molecular weights smaller than 70 kDa have a high tendency to be mobile, and protein subcellular location does not strictly limit protein trafficking.

To determine whether there are certain sequence motifs can be enriched from the mobile proteins, all the sequences of inter-plant mobile proteins were used for the analysis with the Multiple EM for Motif Elicitation (MEME) program (Bailey et al., 2006). However, no sequence motifs were found to be enriched.

Discussion

Plant phloem translocation stream carries numerous mRNAs and proteins, and some of these mobile mRNAs and proteins may play a role in regulating the growth and development in the systemic tissues (Turnbull and Lopez-Cobollo, 2013). Moreover, stress-induced mobile

signals control the systemic responses, which are important for plant adaptation to stresses. For example, in response to nitrogen starvation, plants produce peptides CEPs (C-terminally encoded peptides) in roots, which are translocated to shoot to induce N starvation responses, and the leaf-originated peptide CEPD (CEP downstream) is another mobile signal that travels to roots and plays important roles in nitrogen systemic signaling (Ohkubo et al., 2017; Tabata et al., 2014). Grafting is common practice in agriculture and often the phenotypes of scions (such as pathogen resistance) are influenced by the rootstocks and vice versa (Warschefsky et al., 2016), indicating that the mobile mRNAs, proteins, and other signals travel across the graft junctions and influence the physiology of the systemic tissues (Hettenhausen et al., 2017; Kim et al., 2014; Liu and Chen, 2018; Shahid et al., 2018). Here, we show that various proteins are able to traffic between host plants and dodder and even between different hosts through dodder bridges, and the mobile foreign proteins represent substantial portion (quantitatively, a few to more than 10%) of the recipient plants' proteomes. The long-distance mobile proteins can even be transferred into the foreign plants' seeds.

The following two lines of evidence strongly support the notion that some of the mobile proteins still have biological functions after long-distance translocation: 1) Using transgenic hosts expressing eGFP-GUS, GUS, LUC, PAT, and EPSPS, we demonstrate that these proteins retained their activity after inter-plant movement; 2) importantly, although most mobile proteins' abundance strongly decreased in the foreign plants, we still found that at least 12% of the mobile proteins retained at least 50% of their original abundance, and some proteins even exhibited elevated levels, and some were undetected in the native plants but were identified in the foreign plants. Given that dodder has a very wide host range (across many plant families), proteins with the same functions can be exchanged or transferred, as well as lineage-specific proteins with novel functions can be transferred to recipients through haustorial connections, between dodder and hosts and among phylogenetically distant host plants. Some of these inter-plant mobile proteins may function in the recipient plants by modulating their physiology and may even function in their seeds, such as conferring stress resistance and altering growth and development of subsequent generations.

Venn diagram analysis (Figure 1D) indicated that in addition to the mobile proteins from dodder that are common to the mobile proteins from one or more other species, 148 mobile proteins are dodder-specific. Importantly, some of these dodder-specific mobile proteins may be essential for establishment and/or maintenance of parasitism. Many plant pathogens secrete effector proteins into plant cells to suppress the host plant defense (Deslandes and Rivas, 2012). Similarly, studies on the secretomes of malaria parasites (*Plasmodium falciparum*) also indicated that the secreted proteins remodel the host red blood cell for the survival of these parasites (Hiller et al., 2004; Marti et al., 2004). Whether these dodder-specific long-distance mobile proteins, which could be a part of the dodder parasite secretomic proteins, have functions in manipulating host physiology remains to be determined. Although the mechanism remains unclear, artificial grafting can only be done between plants from the same family, and plants from different families are incompatible (Goldschmidt, 2014), dodder can parasitize plants from various families. It is conceivable that certain secreted dodder proteins function at the haustorium-host junctions and probably other host tissues to enable dodder to form stable graft with the host stem, without inducing rejection reactions in the hosts.

Previously, RNA-seq analysis indicated that more than 9000 and 8000 unigenes from Arabidopsis and dodder (*C. campestris*) were identified in dodder and Arabidopsis stems, respectively; however, between tomato and dodder, there were only 347 and 288 mobile unigenes (Kim et al., 2014), and 2110 mRNAs were found to be transported from Arabidopsis into dodder *C. reflexa* (Thieme et al., 2015). Our RNA-seq analysis on the Arabidopsis-dodder (*C. australis*) system indicated that in Arabidopsis and dodder there were only 1416 and 172 foreign mobile mRNAs; similarly, in the soybean-dodder system, only 64 soybean and 708 dodder mRNAs were found in dodder and soybean, respectively (Supplemental Data 3). The relatively large differences between the numbers of mobile mRNAs in the Arabidopsis-dodder systems identified the previous (Kim et al., 2014; Thieme et al., 2015) and this study may be resulted from the different dodder species (*C. campestris*, *C. reflexa*, and *C. australis*) and different stages of plants. Importantly, we found that the ratios of mobile mRNAs to the total mRNAs were much lower than the ratios of mobile

proteins to the total proteins. For example, in our Arabidopsis-dodder system, only 0.06% of Arabidopsis stem transcriptome reads were from dodder mRNAs, while 4.3% of Arabidopsis stem proteomic signals were from dodder proteins. Furthermore, the distribution patterns of the foreign proteins' ERPA and foreign mRNAs' CNs indicated that the majorities of the foreign proteins exhibited much greater abundance than did the foreign mRNAs. Lastly, in the foreign plants very little mobile proteins and mRNAs were from the same loci. Recently, it was shown that the m^5C (5-methylcytosine)-modified transcript of *YFP-TCTP1* (*yellow fluorescence protein-translationally controlled tumor protein 1*) moved from shoot to root and was translated in root cells to regulate the root growth; however, YFP protein can be translocated into root cells despite that *YFP* transcripts were undetectable in roots (Yang et al., 2019). Therefore, it is very likely that the majorities of the mobile proteins are not *de novo* synthesized from the transferred mRNAs in the foreign plants, but they are *bona fide* long-distance mobile proteins, which occupy large portions of the foreign plants' proteomes, even though the possibility that small numbers of mobile mRNAs can be translated in the recipient plants cannot be completely ruled out.

It had been proposed that three determinants affect the mobility of proteins: protein expression level, subcellular location, and molecular weight (Paultre et al., 2016). In dodder-host parasitic systems, the proteins with relatively high expression levels showed high tendency to be mobile (Figure 5B), and this is in line with the previous studies, in which mRNAs and proteins with high expression levels tend to be mobile (Calderwood et al., 2016; Liu and Chen, 2018; Paultre et al., 2016; Thieme et al., 2015). Consistent with the scenario that macromolecules with molecular weights below 70 kDa could enter the phloem by default (Paultre et al., 2016; Stadler et al., 2005), the molecular weights of mobile proteins in cucurbit and parasitic systems mainly ranged from 20 to 70 kDa (Figure 5C, 5D, and Supplemental Figure 5A). The inter-plant mobile proteins were predicted to be mainly localized in chloroplast, nucleus, cytoplasm, and mitochondrion (Figure 5E and Supplemental Table 6), reminiscent of the subcellular location patterns of the mobile proteins detected within plants (Batailler et al., 2012; Paultre et al., 2016). Furthermore, a small fraction of mobile proteins were predicted to be located at ER or Golgi apparatus (Figure 5E and

Supplemental Table 6), providing new evidence arguing against the notion that proteins targeted to ER and Golgi apparatus are unable to enter translocation stream (Paultre et al., 2016), and this was also supported by the predicted subcellular locations of the phloem sap proteins in cucurbits, in which ER- and Golgi apparatus-localized proteins were also identified (Supplemental Figure 4B and Supplemental Table 6). MEME analysis on the large number of inter-plant mobile proteins resulted in no enrichment of protein sequence motifs, supporting the notion that proteins enter the phloem translocation stream by default, but not by design (Paultre et al., 2016). However, the new finding that mRNAs harboring m^5C mRNA modifications are likely to be mobile (Yang et al., 2019) suggests that certain post-translational modifications might contribute to protein mobility.

This study reveals that between hosts and dodder, large-scale inter-plant protein transfer may play an important role in the host-dodder and host-dodder-host interaction and adaptation to environmental factors as a whole, adding another means of inter-specific communications, in addition to the previously identified transfer of mRNAs, small RNAs, secondary metabolites, and systemic signals (Hettenhausen et al., 2017; Kim et al., 2014; Shahid et al., 2018). This work also suggests that within a plant, extensive protein transfer likely occurs among different cells, tissues, and organs, and this is probably intrinsically required for the delicate orchestration of growth and development in response to environmental conditions. These data also provide new insights into the mechanisms by which rootstocks influence scion phenotypes (Warschefsky et al., 2016).

METHODS

Plasmid construction and plant transformation

The coding sequences of *eGFP-GUS* (*AWH11767, AAF65404*), *eGFP* (*AWH11767*), and *LUC* (*AFE85520*) were cloned into the binary vector pCAMBIA3301 (containing a *PAT* (*AFA36668*) gene) with two restriction endonucleases Nco I and BstE II. These three

constructs and the unmodified pCAMBIA3301, which contains a *GUS* (*AAF65404*) gene, were transformed into agrobacterium (GV3101). A 3-kb Arabidopsis *AOS (AT5G42650)* promoter fragment fused with the full-length coding sequence of *AOS-FLAG* was introduced into the binary vector pCAMBIA2301. Arabidopsis (*Arabidopsis thaliana* Col-0) was transformed with GV3101 carrying vectors indicated above as, following a floral dipping method described by Clough and Bent (Clough and Bent, 1998). Transgenic Arabidopsis seedlings were selected on 1/2 MS (Murashige and Skoog) medium supplemented with 50 mg/L glufosinate or kanamycin.

Plant growth and sampling

Arabidopsis plants (wild-type and transgenic lines) were cultivated on Jiffy peat pellets with diameter of 3.5 cm and height of 4 cm (Jiffy; www.jiffygroup.com) under short day condition (8 h light, 16 h dark) at 22 °C for one month and then individually transferred into pots (16 cm in diameter and 14 cm in height) in a glasshouse, under natural sunlight (~ 12 h light, 12 h dark) at 18-24 °C. Soybean (*Glycine max* Williams 82) and wild tomato (*Solanum pennelli*) were grown in another glasshouse under natural light (~ 12 h light, 12 h dark) at 22-28 °C. Dodder (*Cuscuta australis*) seeds were immersed into sulfuric acid for 30 min, followed by 10 times of rinsing with water (Li et al., 2015). 4-day-old dodder seedlings were infested on 4-week-old wild tomato plants to obtain vigorously growing dodder stocks.

To investigate protein and mRNA trafficking between dodder and hosts, 7-day-bolted Arabidopsis and 2-week-old soybean plants were each infected with a stem segment excised from the dodder stocks. Three weeks after initiation of infestation, Arabidopsis stems, soybean stems, and dodder proximal and distal stem segments were sampled for label-free peptide quantitation proteome analyses. Arabidopsis, soybean, and dodder stems were sampled for transcriptome analyses (the proximal and distal segments of dodder stem were equally mixed). The dodder proximal stem segment was a 2-cm stem segment that was 1 cm away from the attachment region. The dodder distal stem segment was a 4-cm segment that was 8 cm away from the attachment region. Arabidopsis and soybean stems were 2-cm long

segments and 2 cm above the top of the attachment regions. Three replicates were used for proteomic and transcriptomic analyses, each of which was pooled from at least 5 stem segments.

To study the protein transfer between different hosts through dodder connections, 7-day-bolted Arabidopsis and 2-week-old soybean plants were infected with stem segments excised from dodder stocks. These dodder-infested Arabidopsis and soybean plants were placed in pairs closely (~ 10 cm apart). The newly emerged and elongated dodder vines from each Arabidopsis and soybean pair were used to parasitize each other. In two weeks, Arabidopsis-dodder-soybean plant clusters were formed. The three youngest rosette leaves of each Arabidopsis and the youngest trifoliate of each soybean were sampled for proteome. Three replicates were used for proteomic analyses, each of which was pooled from leaves collected from at least five plants.

To obtain dodder and soybean seeds from soybean-dodder parasitization systems, 2-week-old soybean plants were infected with young dodder segments excised from stocks to form soybean-dodder parasitization system. This system was cultivated under normal glasshouse conditions (~12h light, 12h dark) at 22-28 °C until both produced seeds. When the seeds were mature enough, they were collected and dried in a desiccator, before being processed for proteome and transcriptome sequencing. Three biological replicates were used for proteomic and transcriptomic analysis, each of which was pooled from seeds collected from three individual plants.

Protein extraction and digestion for proteome

Samples (each had three biological replicates) were grounded in liquid nitrogen. Lysis buffer (7 M urea, 2 M thiourea, 0.1% CHAPS, protease inhibitor) were added into each sample, and samples were vortexed and ultrasonically disrupted to extract the total proteins. Protein concentrations were determined using the Bradford method (Bradford, 1976). Twenty micrograms of protein of each sample was loaded onto sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie blue. Protein gels were destained until the backgrounds were totally clear, and each lane was divided into three gel fractions according to the protein concentrations. Each gel fraction was further sliced into 1-mm^2 pieces and were washed with the destaining buffer (50 mM NH_4HCO_3 : ACN = 1 : 1). Next, the destained gel fraction pieces were digested with 0.01 μg/μL trypsin working buffer (trypsin was dissolved in 25 mM NH_4HCO_3) overnight at 37 °C. Supernatants were collected and frozen to dehydrate under a vacuum condition.

HPLC-MS

The dehydrated peptides were dissolved in 20 μL of 2% methanol containing 0.1% formic acid, after centrifugation, 10 μL of these dissolved digested peptides were loaded onto the ThermoFisher Scientific EASY-nLC 1000 System (Nano HPLC) with a loading pump at the speed of 3 μL/min for 8 min. Then, peptide mixtures were separated with two mobile phases: solution A (100% ultrapure water and 0.1% formic acid) and solution B (100% acetonitrile and 0.1% formic acid) at a flow rate of 300 nL/min. The separation gradient was as follows: 3% solution B for 5 min, 8% for 102 min, 28% for 3 min, 80% for 10 min, and then back to initial condition. The separated peptides were loaded into an Orbitrap Fusion mass spectrometer (ThermoFisher Scientific) through a nano-ESI emitter with the spray voltage of the ion source of 2.4 kV and the capillary temperature of 320 °C. Full-scan mass spectra were acquired in the MS over 300-1500 m/z with a resolution of 120000, automatic gain control (AGC) target was 2.0e4 and maximum injection time was set to 50 ms. All ions were selected for high energy collision induced dissociation (HCD), the normalization collision energy was set to 30%. HCD scans were collected at 30000 resolution, AGC target was set to 1.0e4, and maximum injection time was 45 ms.

Data processing for proteomics

The MS raw data were processed using MaxQuant software. Parameters of MaxQuant searches were set as follows: precursor ion mass tolerance was 15 ppm, fragment ion mass

tolerance was 20 mmu, and the max missed cleavages was 2. The processed MS data were searched against the uniport Arabidopsis and soybean database (https://www.uniprot.org/) and dodder database (http://www.dodderbase.org/) to identify the proteins.

For each group of samples, only peptides that appeared in at least two out of three replicates were chosen for further analyses. The sequences of identified peptides were mapped to the dodder and host (Arabidopsis or soybean) genome, if any did not match either genome (because of sequencing errors and posttranslational modifications, etc.), they were filtered out. Mobile proteins were then filtered using the following criterion: According to the sample origin, proteins that only matched the database of foreign plants, but did not match the database of the original plant, were picked out as mobile proteins; proteins which matched both the databases of original and foreign plants were considered to be the original proteins.

Venn diagram analyses were carried out using an online platform (http://bioinfogp.cnb.csic.es/tools/venny/). GO term enrichments were run on the RStudio loaded with the GO package, and the false discovery rate (FDR) was set to 0.05. To estimate the proportions of mobile proteins in a given proteome, the total precursor intensities (TPIs) of mobile proteins were divided by the TPIs of all expressed proteins. Subcellular locations of mobile proteins were predicted according to their sequence properties using an online platform (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/) in the Plant-mPLoc module.

To identify gene families in Arabidopsis, dodder, soybean, cucumber, and watermelon, we first obtained protein sequences of those species. We obtained proteins of *Arabidopsis thaliana* (TAIR10) from TAIR database (Lamesch et al., 2012), and proteins sequences of *Cucumis sativus* L. var. sativus cv. 9930 (v2) and *Citrullus lanatus* subsp. vulgaris cv. 97103 (v1) from CuGenDB (Zheng et al., 2019). Proteins of *Cuscuta australis* (v1.1) were obtained from (http://dodderbase.org/) (Sun et al., 2018), and proteins of *Glycine max* were retrieved from Uniprot (UniProt Consortium, 2018). We then performed all-vs-all blast using ncbi-blast+ (v2.2.28) (Altschul et al., 1990) with protein sequences from those five species.

Gene pairs with blast scores were converted into a graph and gene families were inferred using OrthoFinder2 (v2.2.6) (Emms and Kelly, 2018) with inflation parameter set to 1.5.

Transcriptome analysis

Samples (each had three biological replicates) were grounded in liquid nitrogen. Each sample was initially treated with Fruit-Mate for RNA purification (Takara) following the manufacturer's instructions, and thereafter the total RNAs were isolated with TRIzol reagent (ThermoFisher Scientific). Illumina TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA, USA) was used to build cDNA libraries. Generated cDNA libraries were sent to a HiSeq2500-PE125 platform (Illumina) to acquire sequence reads. In soybean-dodder system, more than 15.3, 18.9, 16.2 and 14.7 Gb clean aligned pairs were obtained from dodder stem, soybean stem, dodder seed and soybean seed samples, respectively. In Arabidopsis-dodder system, more than 14.9 and 20.6 Gb clean aligned pairs were obtained from dodder stem and Arabidopsis stem samples, respectively. Raw data of soybean samples were mapped to soybean genome first and the unmapped reads were matched against dodder genome. Thus, the resulting dodder sequences were identified as mobile dodder mRNAs. Mobile Arabidopsis and soybean mRNAs were similarly filtered. Mobile Arabidopsis, dodder, and soybean mRNAs with more than four count numbers per transcript were picked up for future analyses.

Western blotting

Plant tissues (~100 mg) were grounded in liquid nitrogen and 200 μL of protein extraction buffer (100 mM Hepes, pH 7.5, 5 mM EGTA, 5 mM EDTA, 10 mM DTT, 1 mM PMSF, 10% glycerol, Proteinase Inhibitor Cocktail (Sigma)) were added into each sample to extract total proteins. Protein concentrations were determined using the Bradford method (Bradford, 1976). For the detection of eGFP, 5 μg Arabidopsis and 15 μg dodder total proteins were used. For the detection of AOS-FLAG, 20 μg of dodder total proteins were loaded. Proteins were electroblotted onto a polyvinylidene fluoride (PVDF) membrane using a wet blotting

method (Bio-Rad). The membrane was first stained with Ponceau S and scanned, and after destaining, membrane was blocked 2 h with 5% not-fat milk, followed by 2 h of incubation with rabbit polyclonal Anti-GFP antibody (1:1000, Abcam) and Anti-FLAG antibody (1:1000, Sigma) and 2 h of incubation with secondary HRP antibody which was raised against rabbit antibodies (ThermoFisher Scientific). Exactly the same amounts of proteins were used for immuno-detection of actin using an anti-actin (Abmart) antibody. The imaging was done on a chemiluminescence gel imager system (MicroChemi, DNR).

LUC activity assay

For imaging LUC activity, stem segments (~ 5 cm), which were the dodder attachment regions, were excised from Arabidopsis WT and a transgenic line expressing LUC. D-Luciferin (1 mM, in 0.1% Triton X-100) was sprayed to the Arabidopsis stem segments and dodder stems. After 5 min, the whole Arabidopsis stem segment with dodder was placed onto the imaging platform and the bright light and chemiluminescence images were taken on a chemiluminescent imaging system (Tanon).

For quantitative analysis on the LUC activity, samples were grounded in liquid nitrogen and LUC activity was measured using a Luciferase Assay System kit following the kit instructions (Promega). Water was measured as the background.

GUS staining and light microscopy

Dodder stems that collected from eGFP-GUS and WT Arabidopsis were embedded into 3% low-melting-point agarose, and cross sections were cut into 70-90 μm thickness on a vibratome (Leica, VT1200S). Cross sections were stained with a GUS staining solution (1 mg/mL X-Gluc, 100 mM phosphate buffer, pH 7.0, 0.5 mM $K_3[Fe(CN)_6]$, 0.5 mM $K_4[Fe(CN)_6]$, 10 mM EDTA) at 37 ^{o}C overnight. Sections were washed three times with pure water to remove GUS working solution, and images were taken on a light microscope (Lecia, DM55008).

692

693 **GUS activity assay**

694

695 Samples were grounded in liquid nitrogen and total proteins were extracted as described
696 above. Quantitative fluorometric GUS assays were carried out following a GUS assay
697 protocol that described by Jefferson et al. (Jefferson et al., 1987). Water was used in GUS
698 activity assay as the background.

699

700 **Glufosinate tolerance of dodder stems**

701

702 Dodders were infested on WT and transgenic Arabidopsis which overexpressed PAT. Three
703 weeks after the infestation, dodder stems were painted with 0.72 g/L glufosinate solution
704 (containing 0.1% Triton X-100) with a soft paintbrush. Seven days after the treatment, these
705 dodder stems were collected and photographed.

706

707 **Glyphosate tolerance of dodder seeds**

708

709 Dodder seeds were harvested form WT soybean and soybean expressing EPSPS,
710 respectively. Seeds were scarified in concentrated sulphuric acid for 30 min and immediately
711 wash with water thoroughly. Thirty dodder seeds were treated with 5 mg/mL glyphosate
712 solution (containing 0.1% Triton X-100) or water, and the germination rates were recorded 5
713 days later.

714

715 **RNA isolation and RT-PCR**

716

717 To quantify the transcripts of *eGFP-GUS*, *GUS*, *LUC*, *eGFP*, and *PAT* in dodder, 50-100 mg
718 dodder stems that collected from dodder parasitizing on transgenic and WT Arabidopsis were
719 ground in liquid nitrogen. Total RNAs were isolated using TRIzol (ThermoFisher Scientific),
720 and 0.5 μg of total RNA from each sample was reverse transcribed using oligo(dT) and

Superscript II reverse transcriptase (ThermoFisher Scientific). PCRs were run in 35 cycles and primers used are listed in Supplementary Table 7.

Data availability

The original sequencing data of transcriptome were available at the NCBI under the BioProject PRJNA528603, PRJNA528604, PRJNA544568, and PRJNA544643.

Author contributions

Conceptualization, J.W.; Investigation, N.L., G.S., J.Z., S.L., J.L., C.Z., J.Q., and L.W.; Formal Analysis, N.L., H.L., and Y.X.; Writing, N.L. and J.W.

Acknowledgments

We thank Dr. Dawei Yan (University of Cambridge) for comments and Dr. Huixia Shou (Zhejiang University) for providing the transgenic soybean seeds expressing GUS and EPSPS. Canrong Ma is thanked for providing technical support in this project. This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB11050200), the National Science Foundation of China (31970274), CAS Youth Innovation Promotion Association (2018426), and the International Partnership Program of the Chinese Academy of Sciences (151853KYSB20170025). The authors declare no competing interests.

REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J Mol Biol 215:403-410.

Bailey, T.L., Williams, N., Misleh, C., and Li, W.W. (2006). MEME: discovering and analyzing DNA and protein sequence motifs. Nucleic Acids Res 34:W369-W373.

Batailler, B., Lemaitre, T., Vilaine, F., Sanchez, C., Renard, D., Cayla, T., Beneteau, J., and Dinant, S. (2012). Soluble and filamentous proteins in *Arabidopsis* sieve elements. Plant Cell Environ 35:1258-1273.

Birschwilks, M., Haupt, S., Hofius, D., and Neumann, S. (2006). Transfer of phloem-mobile substances from the host plants to the holoparasite *Cuscuta* sp. J Exp Bot 57:911-921.

753 Birschwilks, M., Sauer, N., Scheel, D., and Neumann, S. (2007). *Arabidopsis thaliana* is a susceptible host plant
754 for the holoparasite *Cuscuta* spec. Planta 226:1231-1241.
755 Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein
756 utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.
757 Calderwood, A., Kopriva, S., and Morris, R.J. (2016). Transcript Abundance Explains mRNA Mobility Data in
758 *Arabidopsis thaliana*. Plant Cell 28:610-615.
759 Chou, K.-C., and Shen, H.-B. (2010). Cell-PLoc 2.0: an improved package of web-servers for predicting
760 subcellular localization of proteins in various organisms. Natural Science 02:1090-1103.
761 Clarke, C.R., Timko, M.P., Yoder, J.I., Axtell, M.J., and Westwood, J.H. (2019). Molecular Dialog Between
762 Parasitic Plants and Their Hosts. Annu Rev Phytopathol 57:1–21.
763 Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation
764 of *Arabidopsis thaliana*. Plant J 16:735-743.
765 Deslandes, L., and Rivas, S. (2012). Catch me if you can: bacterial effectors and plant targets. Trends Plant Sci
766 17:644-655.
767 Du, B., Wei, Z., Wang, Z.Q., Wang, X.X., Peng, X.X., Du, B., Chen, R.Z., Zhu, L.L., and He, G.C. (2015).
768 Phloem-exudate proteome analysis of response to insect brown plant-hopper in rice. J Plant Physiol
769 183:13-22.
770 Emms, D.M., and Kelly, S. (2018). OrthoFinder2: fast and accurate phylogenomic orthology analysis from gene
771 sequences. bioRxiv.
772 Goldschmidt, E.E. (2014). Plant grafting: new mechanisms, evolutionary implications. Front Plant Sci 5.
773 Haupt, S., Oparka, K.J., Sauer, N., and Neumann, S. (2001). Macromolecular trafficking between *Nicotiana*
774 *tabacum* and the holoparasite *Cuscuta reflexa*. J Exp Bot 52:173-177.
775 Hettenhausen, C., Li, J., Zhuang, H.F., Sun, H.H., Xu, Y.X., Qi, J.F., Zhang, J.X., Lei, Y.T., Qin, Y., Sun, G.L., et
776 al. (2017). Stem parasitic plant *Cuscuta australis* (dodder) transfers herbivory-induced signals among
777 plants. P Natl Acad Sci USA 114:E6703-E6709.
778 Hiller, N.L., Bhattacharjee, S., van Ooij, C., Liolios, K., Harrison, T., Lopez-Estrano, C., and Haldar, K. (2004).
779 A host-targeting signal in virulence proteins reveals a secretome in malarial infection. Science
780 306:1934-1937.
781 Hosford, R.M. (1967). Transmission of Plant Viruses by Dodder. Bot Rev 33:387-406.
782 Hu, C.Y., Ham, B.K., El-Shabrawi, H.M., Alexander, D., Zhang, D.B., Ryals, J., and Lucas, W.J. (2016).
783 Proteomics and metabolomics analyses reveal the cucurbit sieve tube system as a complex metabolic
784 space. Plant J 87:442-454.
785 Jefferson, R.A., Kavanagh, T.A., and Bevan, M.W. (1987). GUS fusions: *β*-glucuronidase as a sensitive and
786 versatile gene fusion marker in higher plants. Embo J 6:3901-3907.
787 Jiang, L.J., Qu, F., Li, Z.H., and Doohan, D. (2013). Inter-species protein trafficking endows dodder (*Cuscuta*
788 *pentagona*) with a host-specific herbicide-tolerant trait. New Phytol 198:1017-1022.
789 Kaminska, M., and Korbin, M. (1999). Graft and dodder transmission of phytoplasma affecting lily to
790 experimental hosts. Acta Physiologiae Plantarum 21:21-26.
791 Kim, G., LeBlanc, M.L., Wafula, E.K., Depamphilis, C.W., and Westwood, J.H. (2014). Genomic-scale
792 exchange of mRNA between a parasitic plant and its hosts. Science 345:808-811.
793 Kim, M., Canio, W., Kessler, S., and Sinha, N. (2001). Developmental changes due to long-distance movement
794 of a homeobox fusion transcript in tomato. Science 293:287-289.
795 Lamesch, P., Berardini, T.Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Muller, R., Dreher, K., Alexander,
796 D.L., Garcia-Hernandez, M., et al. (2012). The Arabidopsis Information Resource (TAIR): improved

gene annotation and new tools. Nucleic Acids Res 40:D1202-1210.

Li, J., Hettenhausen, C., Sun, G., Zhuang, H., Li, J.H., and Wu, J. (2015). The parasitic plant *Cuscuta australis* is highly insensitive to abscisic acid-induced suppression of hypocotyl elongation and seed germination. PloS one 10:e0135197.

Liu, L., and Chen, X. (2018). Intercellular and systemic trafficking of RNAs in plants. Nat Plants 4:869-878.

Lopez-Cobollo, R.M., Filippis, I., Bennett, M.H., and Turnbull, C.G.N. (2016). Comparative proteomics of cucurbit phloem indicates both unique and shared sets of proteins. Plant J 88:633-647.

Lough, T.J., and Lucas, W.J. (2006). Integrative plant biology: Role of phloem long-distance macromolecular trafficking. Annu Rev Plant Biol 57:203-232.

Lucas, W.J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S.R., Helariutta, Y., He, X.Q., Fukuda, H., Kang, J., Brady, S.M., et al. (2013). The plant vascular system: evolution, development and functions. Journal of integrative plant biology 55:294-388.

Marti, M., Good, R.T., Rug, M., Knuepfer, E., and Cowman, A.F. (2004). Targeting malaria virulence and remodeling proteins to the host erythrocyte. Science 306:1930-1933.

Ohkubo, Y., Tanaka, M., Tabata, R., Ogawa-Ohnishi, M., and Matsubayashi, Y. (2017). Shoot-to-root mobile polypeptides involved in systemic regulation of nitrogen acquisition. Nat Plants 3:17029-17034.

Ostendorp, A., Pahlow, S., Krussel, L., Hanhart, P., Garbe, M.Y., Deke, J., Giavalisco, P., and Kehr, J. (2017). Functional analysis of *Brassica napus* phloem protein and ribonucleoprotein complexes. New Phytol 214:1188-1197.

Paultre, D.S.G., Gustin, M.P., Molnar, A., and Oparka, K.J. (2016). Lost in transit: Long-distance trafficking and phloem unloading of protein signals in Arabidopsis homografts. Plant Cell 28:2016-2025.

Shahid, S., Kim, G., Johnson, N.R., Wafula, E., Wang, F., Coruh, C., Bernal-Galeano, V., Phifer, T., dePamphilis, C.W., Westwood, J.H., et al. (2018). MicroRNAs from the parasitic plant *Cuscuta campestris* target host messenger RNAs. Nature 553:82-85.

Smith, J.D., Woldemariam, M.G., Mescher, M.C., Jander, G., and De Moraes, C.M. (2016). Glucosinolates from host plants influence growth of the parasitic plant *Cuscuta gronovii* and its susceptibility to aphid feeding. Plant physiology 172:181-197.

Stadler, R., Wright, K.M., Lauterbach, C., Amon, G., Gahrtz, M., Feuerstein, A., Oparka, K.J., and Sauer, N. (2005). Expression of GFP-fusions in Arabidopsis companion cells reveals non-specific protein trafficking into sieve elements and identifies a novel post-phloem domain in roots. Plant J 41:319-331.

Sun, G.L., Xu, Y.X., Liu, H., Sun, T., Zhang, J.X., Hettenhausen, C., Shen, G.J., Qi, J.F., Qin, Y., Li, J., et al. (2018). Large-scale gene losses underlie the genome evolution of parasitic plant *Cuscuta australis*. Nat Commun 9:2683-2695.

Tabata, R., Sumida, K., Yoshii, T., Ohyama, K., Shinohara, H., and Matsubayashi, Y. (2014). Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. Science 346:343-346.

Thieme, C.J., Rojas-Triana, M., Stecyk, E., Schudoma, C., Zhang, W., Yang, L., Minambres, M., Walther, D., Schulze, W.X., Paz-Ares, J., et al. (2015). Endogenous *Arabidopsis* messenger RNAs transported to distant tissues. Nat Plants 1:15025-15032.

Turck, F., Fornara, F., and Coupland, G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu Rev Plant Biol 59:573-594.

Turnbull, C.G.N., and Lopez-Cobollo, R.M. (2013). Heavy traffic in the fast lane: long-distance signalling by macromolecules. New Phytol 198:33-51.

UniProt Consortium, T. (2018). UniProt: the universal protein knowledgebase. Nucleic Acids Res 46:2699.

Vogel, A., Schwacke, R., Denton, A.K., Usadel, B., Hollmann, J., Fischer, K., Bolger, A., Schmidt, M.H., Bolger, M.E., Gundlach, H., et al. (2018). Footprints of parasitism in the genome of the parasitic flowering plant *Cuscuta campestris*. Nat Commun 9:2515-2526.

von Malek, B., van der Graaff, E., Schneitz, K., and Keller, B. (2002). The *Arabidopsis* male-sterile mutant *dde2-2* is defective in the *ALLENE OXIDE SYNTHASE* gene encoding one of the key enzymes of the jasmonic acid biosynthesis pathway. Planta 216:187-192.

Warschefsky, E.J., Klein, L.L., Frank, M.H., Chitwood, D.H., Londo, J.P., von Wettberg, E.J.B., and Miller, A.J. (2016). Rootstocks: Diversity, Domestication, and Impacts on Shoot Phenotypes. Trends Plant Sci 21:418-437.

Westwood, J.H., Yoder, J.I., Timko, M.P., and dePamphilis, C.W. (2010). The evolution of parasitism in plants. Trends Plant Sci 15:227-235.

Winter, N., and Kragler, F. (2018). Conceptual and methodological considerations on mRNA and proteins as intercellular and long-distance signals. Plant Cell Physiol 59:1700-1713.

Wu, J.Q., Hettenhausen, C., Meldau, S., and Baldwin, I.T. (2007). Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of Nicotiana attenuata. Plant Cell 19:1096-1122.

Yang, L., Perrera, V., Saplaoura, E., Apelt, F., Bahin, M., Kramdi, A., Olas, J., Mueller-Roeber, B., Sokolowska, E., Zhang, W., et al. (2019). m^5C Methylation Guides Systemic Transport of Messenger RNA over Graft Junctions in Plants. Current Biology 29:1-12.

Zheng, Y., Wu, S., Bai, Y., Sun, H., Jiao, C., Guo, S., Zhao, K., Blanca, J., Zhang, Z., Huang, S., et al. (2019). Cucurbit Genomics Database (CuGenDB): a central portal for comparative and functional genomics of cucurbit crops. Nucleic Acids Res 47:D1128-D1136.

Zhuang, H., Li, J., Song, J., Hettenhausen, C., Schuman, M.C., Sun, G., Zhang, C., Li, J., Song, D., and Wu, J. (2018). Aphid (*Myzus persicae*) feeding on the parasitic plant dodder (*Cuscuta australis*) activates defense responses in both the parasite and soybean host. New Phytol 218:1586-1596.

867 **FIGURE LEGENDS**

868

869 **Figure 1. Protein transfer between host and dodder stems.** (**A** and **B**) Schematics indicating
870 the experiment setup and the numbers of mobile proteins between dodder and Arabidopsis (**A**)
871 and dodder and soybean (**B**). HS, host stem; DS1, dodder stem proximal segment; DS2, dodder
872 stem distal segment. Red numbers are proteins from hosts; purple numbers are proteins from
873 dodder. (**C**) Venn diagrams indicating the common and specific host proteins detected in
874 proximal and distal segments of dodder stems whose hosts were Arabidopsis or soybean. (**D**)
875 Venn diagram analysis of the long-distance mobile proteins identified in Arabidopsis- and
876 soybean-dodder parasitization systems and the phloem sap proteins identified in cucurbit (Hu
877 et al., 2016). (**E**) The estimated percentages and estimated relative protein abundance (ERPA)
878 of foreign and native proteins in the total proteins of different plant stems. The percentages
879 were estimated based on total precursor intensities (TPIs). ERPA is based on the signal
880 intensities of peptides. Proteins identified in the Arabidopsis-dodder and soybean-dodder
881 systems are shown on the left and right, respectively. The percentages of native and foreign
882 proteins were shown in pie charts, and the ERPA was shown in box plots. The upper and lower
883 edges of box plots indicated the first and third quartiles and the bands and "×" in the boxes were
884 medians and means of data. The whiskers extended to 1.5 times interquartile ranges.

885

886 **Figure 2. Translocation of transgenic proteins from Arabidopsis to dodder.** (**A** to **B**) GUS
887 staining of dodder stem cross sections. Dodder were infested on transgenic Arabidopsis
888 expressing eGFP-GUS (**A**) and wild-type (WT) Arabidopsis (**B**). Cross sections of dodders
889 were stained for visualizing GUS activity (blue spots, one of which is highlighted in a red
890 box). (**C**) GUS activity assay of dodder stems that grew on WT Arabidopsis and transgenic
891 Arabidopsis expressing GUS (denoted as WT and GUS, respectively). Black spots in box plot
892 represented the 9 biological replicates (each replicate was collected from three individual
893 plants). The maximum values, upper quartiles, median, lower quartiles, and minimum values
894 were shown in the box plot. Student's t-test; n = 5; ***, $p < 0.001$. (**D**) Chemiluminescence
895 and bright light images of dodder and hosts (Arabidopsis expressing LUC and WT
896 Arabidopsis, respectively). Red arrows denote LUC activity in dodder stem. (**E**) Western

blotting of eGFP protein in dodder and Arabidopsis host expressing eGFP. Lanes 1, transgenic Arabidopsis expressing eGFP; 2, WT Arabidopsis; 3 and 4, proximal segment of dodder stem parasitizing transgenic Arabidopsis expressing eGFP (3) or WT Arabidopsis (4); 5 and 6, distal segment of dodder stem parasitizing transgenic Arabidopsis expressing eGFP (5) or WT Arabidopsis (6). Note: Five and 15 μg of Arabidopsis and dodder proteins were loaded, respectively. (**F**) Detection of AOS-FLAG in dodder parasitizing Arabidopsis expressing AOS-FLAG. D1, D2, and D3: stems of three individual dodder plants parasitizing transgenic Arabidopsis exressing AOS-FLAG; D control: dodder stems parasitizing WT Arabidopsis. Note: 20 μg of dodder proteins were loaded. Ponceau S staining of Rubisco large subunit (for Arabidopsis) and western blotting of actin (for dodder) were performed as loading reference in (**E**) and (**F**).

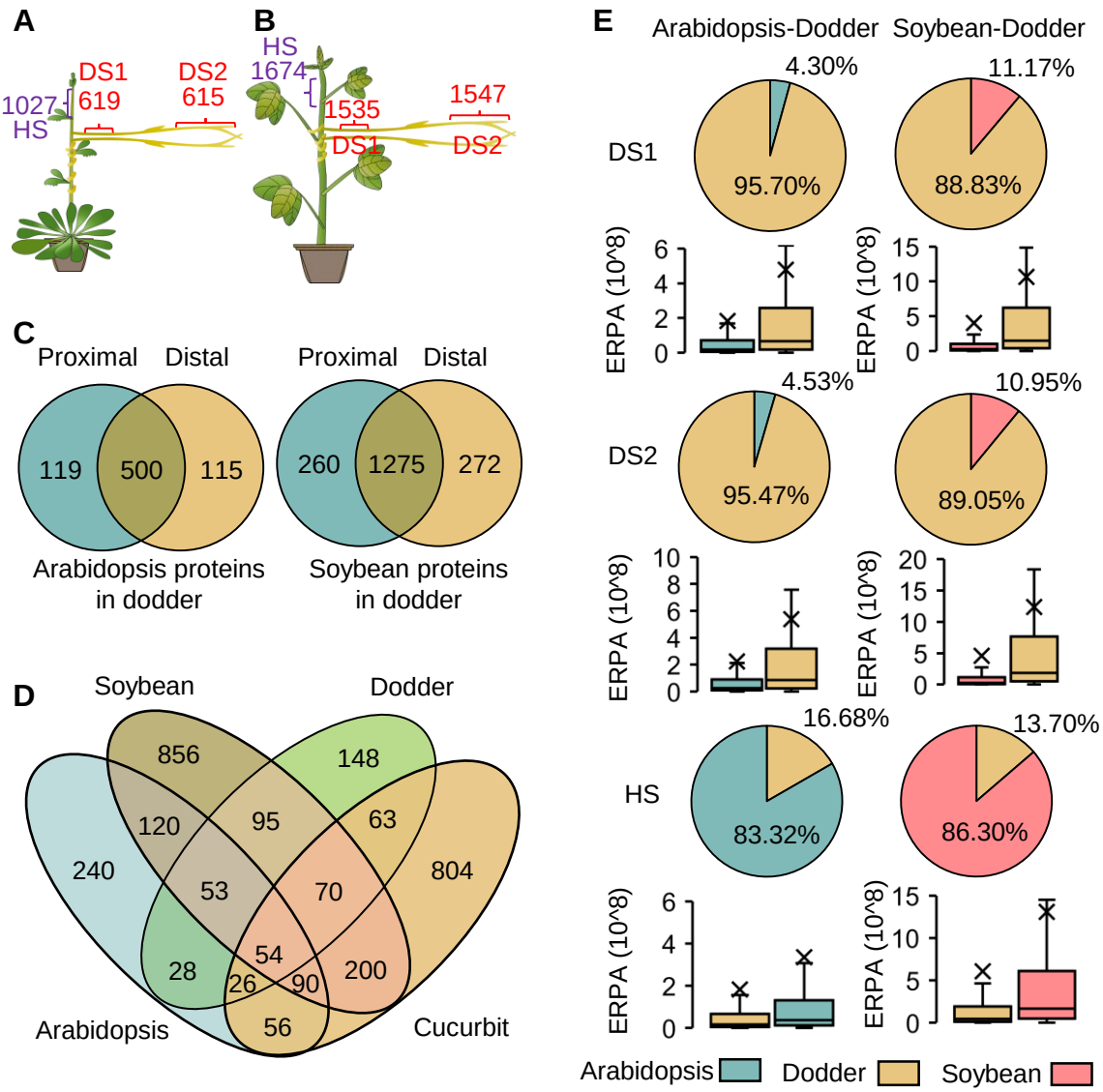
Figure 3. Translocation of inter-plant proteins and mRNAs in soybean and dodder seeds. **(A**) Schematic indicating the experiment setup and the numbers of foreign proteins identified in the soybean seeds (SS) and dodder seeds (DS). (**B**) The estimated percentages and estimated relative protein abundance (ERPA) or count number (CN) of foreign and native proteins and mRNAs in dodder and soybean seed proteome and transcriptome. DS, dodder seeds; SS, soybean seeds. For proteins, calculation was based on the ratio between the total precursor intensities of foreign and total proteins, ERPA was based on the signal intensity of peptides; for mRNAs, calculation was based on the ratio between the reads mapped to the foreign genome and total reads of those mapped to the foreign and native genome, CNs were based on the count number of transcripts. The percentages were shown in pie charts, and the ERPA and CNs were shown in box plots. The upper and lower edges of box plots showed the first and third quartiles, the bands and "×" in the boxes were medians and means of data. The whiskers extended to 1.5 times interquartile range. (**C**) Comparisons of foreign mRNAs and proteins that were co-translocated into dodder and soybean seeds. (**D**) GUS staining of dodder flowers that grew on transgenic GUS soybean and WT soybean, respectively. (**E**) GUS activity in the seeds of dodder grown on transgenic GUS soybean and WT soybean. Student's t-test; n = 6; **, $p < 0.01$. (**F**) Germination rates of seeds of dodders harvested from

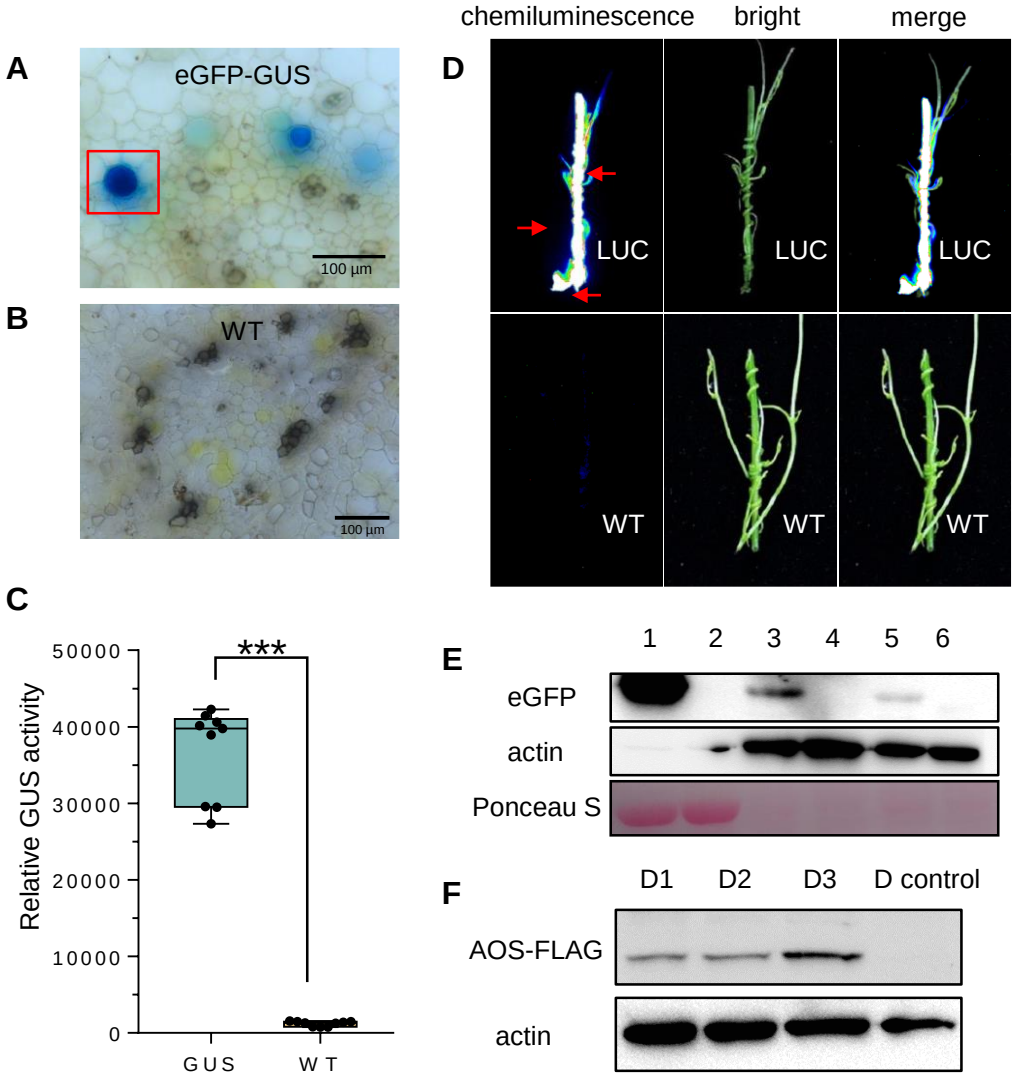
WT and transgenic soybean expressing EPSPS. Student's t-test; n = 5; ***, $p < 0.001$ (each group of seeds were from five individual plants, at least 30 seeds were used for each plant). In (**E**) and (**F**), black spots in box plots represented the 5-6 biological replicates, and the maximum values, upper quartiles, median, lower quartiles, and minimum values were shown in box plots.

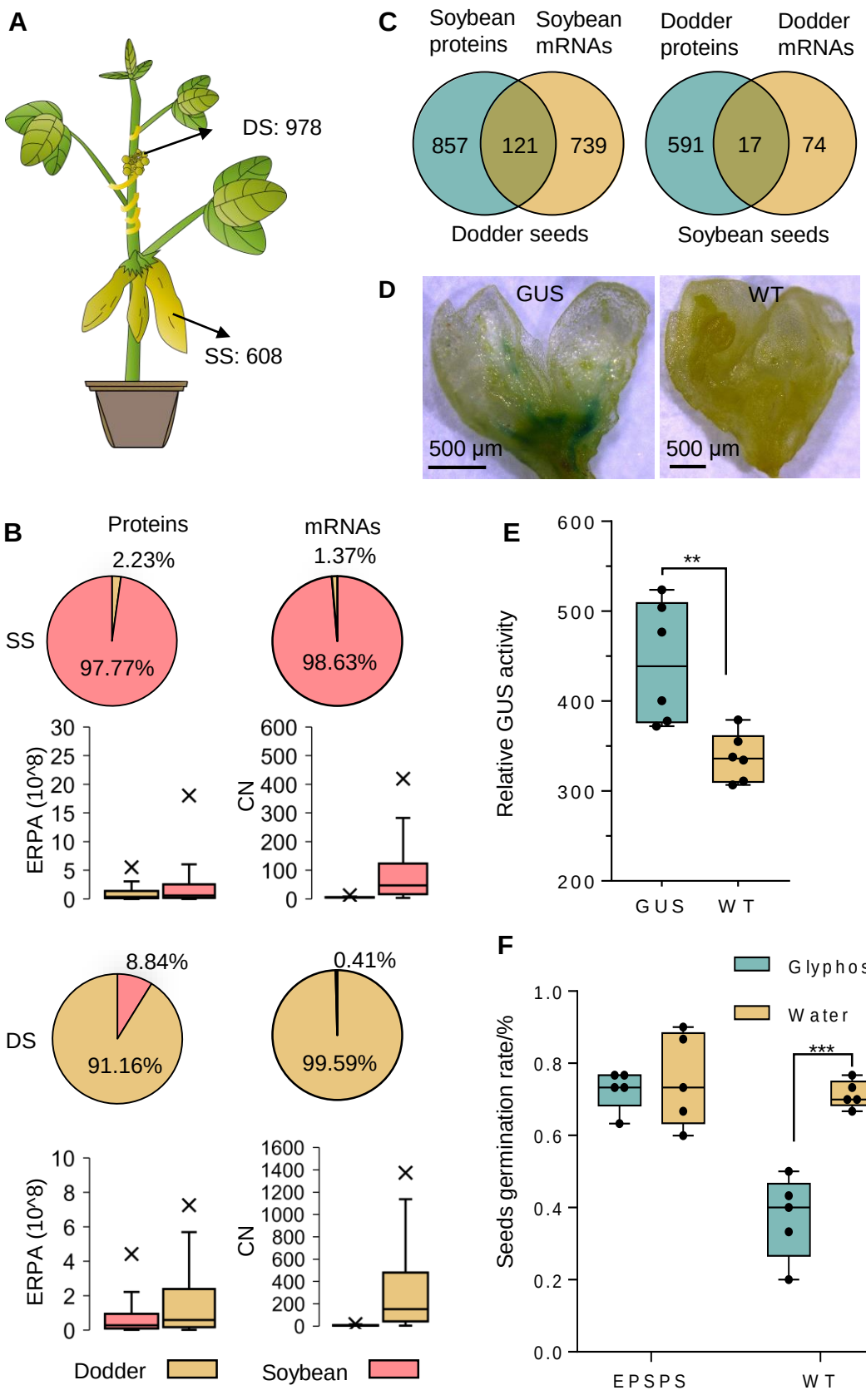
Figure 4. Dodder-mediated inter-host protein transfer. (**A**) Schematic indicating the experiment setup and the numbers of inter-plant mobile proteins detected in Arabidopsis and soybean leaves. AL, Arabidopsis leaves; SL, soybean leaves. (**B**) The percentages and estimated relative protein abundance (ERPA) of foreign host proteins in AL and SL. The percentages were shown in pie charts, and the ERPA was shown in box plots. The upper and lower edges of box plots showed the first and third quartiles, the bands and "×" in the boxes were medians and means of data. The whiskers extended to 1.5 times interquartile range. (**C**) Venn diagram indicating the orthology of the inter-host mobile soybean and Arabidopsis proteins. (**D**) GUS activity in dodder stems and soybean leaves. Soybean was connected with transgenic Arabidopsis expressing eGFP-GUS or with wild-type (WT) Arabidopsis by dodder bridges. Asterisks indicate statistical significance between the two Arabidopsis-dodder-soybean parasitization systems in which Arabidopsis plants expressed eGFP-GUS or were WT. Student's t-test; n = 6; ***, $p < 0.001$. Black spots in box plot represented the 6 biological replicates (each replicate was collected from three individual plants), and the maximum values, upper quartiles, median, lower quartiles, and minimum values were shown in the box plot. (**E**) Photograph of the inflorescence of a *dde2-2* mutant that was bridge connected with a soybean plant by dodder and whose mutant phenotypes were rescued.

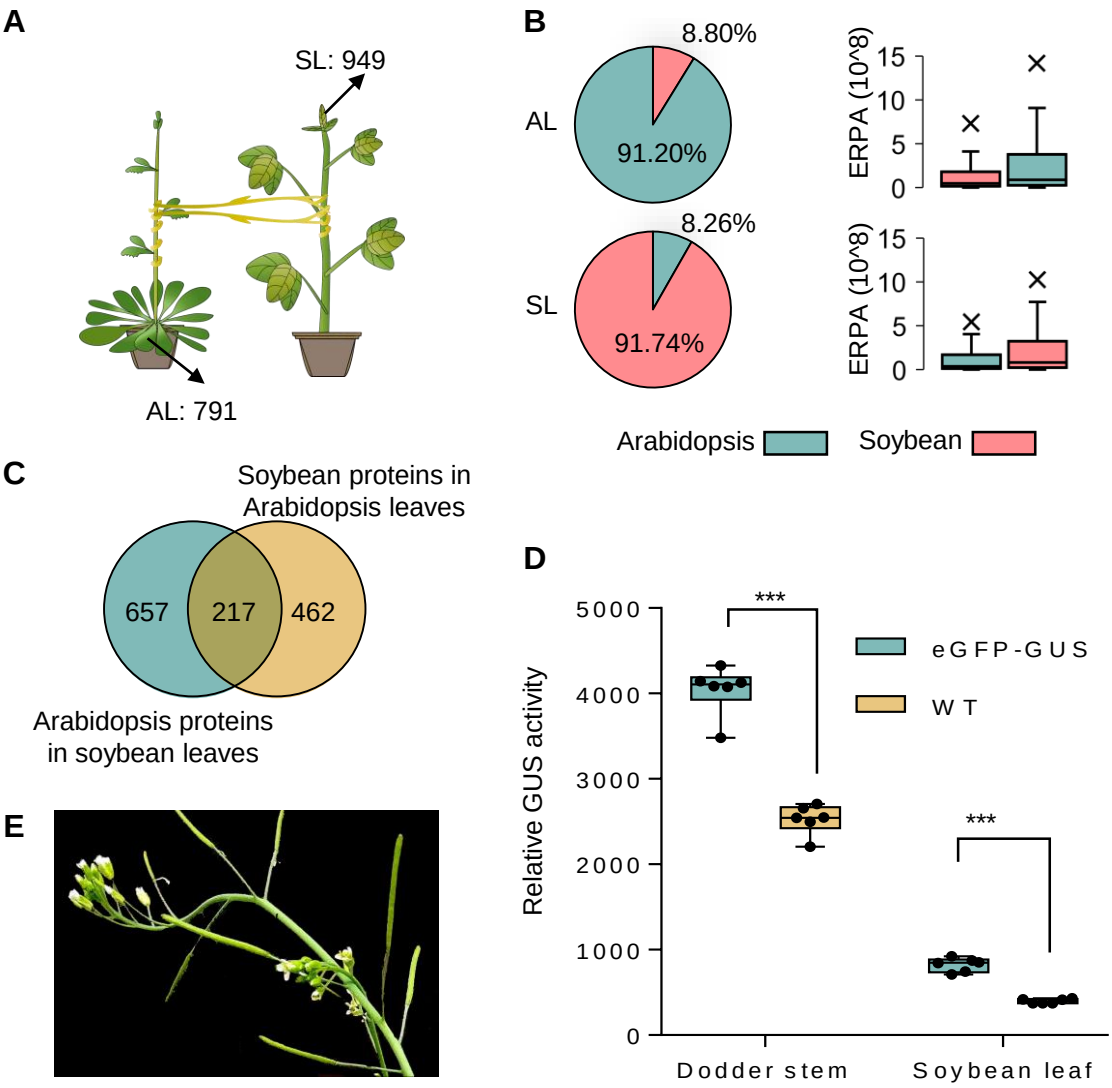
Figure 5. The general properties of mobile proteins. (**A)** Signal intensities of the proteins in foreign and original stems. Arabidopsis (F) and (O) denote Arabidopsis proteins in foreign (dodder)/original (Arabidopsis) stems, respectively; soybean (F) and (O) denote soybean proteins in foreign (dodder)/original (soybean) stems, respectively; dodder (F) and (O) denote dodder proteins in foreign (Arabidopsis and soybean)/original (dodder) stems, respectively.

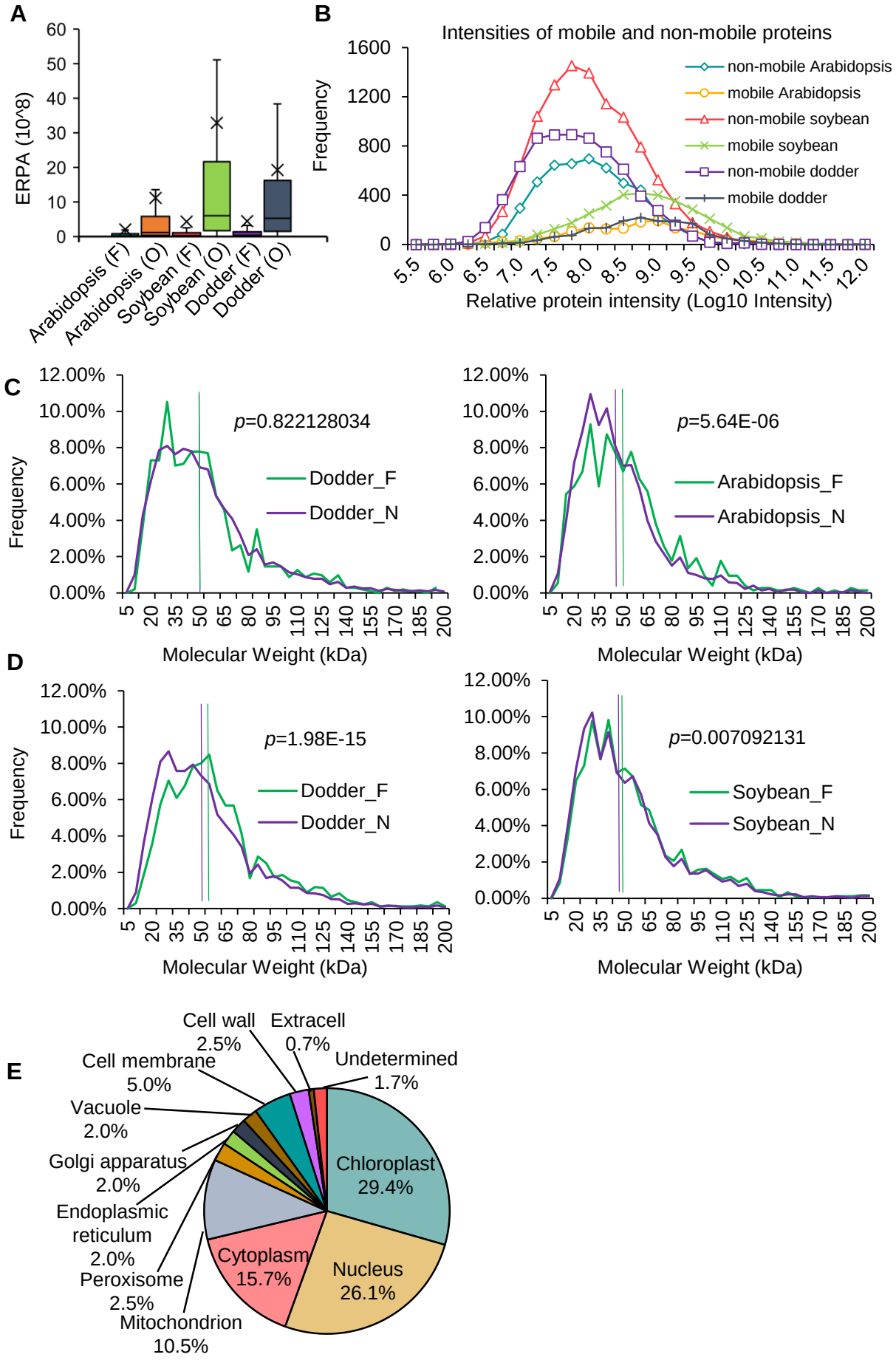
The upper and lower edges of box plots showed the first and third quartiles, the bands and "×" in the boxes were medians and means of data. The whiskers extended to 1.5 times interquartile range. (**B**) Distributions of inter-plant mobile and non-mobile proteins' intensities in source plants. Data are from Figure 1A and 1B. (**C** to **D**) Size distribution of the native (N) proteins and the proteins trafficked to foreign plants (F) detected in Arabidopsis-dodder (**C**) and soybean-dodder (**D**) system. Medians were shown as vertical bars. *p*-value showed the significant difference between the size distribution pattern of foreign and native proteomes (Kolmogorov-Smirnov Test). (**E**) Percentages of the mobile proteins predicted to be located in different subcellular locations. Data are from Figure 1A and 1B.


A
DS1
619
DS2
615
1027
HS
B
HS
1674
1535
DS1
1547
DS2
C
Proximal
Distal
119
500
115
Arabidopsis proteins
in dodder
Proximal
Distal
260
1275
272
Soybean proteins
in dodder
D
Soybean
Dodder
856
148
120
95
63
240
804
53
70
54
28
26
90
200
56
Arabidopsis
Cucurbit
E
Arabidopsis-Dodder
Soybean-Dodder
DS1
4.30%
95.70%
11.17%
88.83%
ERPA (10^8)
DS2
4.53%
95.47%
10.95%
89.05%
HS
16.68%
83.32%
13.70%
86.30%
Arabidopsis
Dodder
Soybean


A
eGFP-GUS
100 µm
B
WT
100 µm
C
Relative GUS activity
50000
40000
30000
20000
10000
0
***
GUS
WT
D
chemiluminescence
bright
merge
LUC
LUC
LUC
WT
WT
WT
E
1 2 3 4 5 6
eGFP
actin
Ponceau S
F
D1 D2 D3 D control
AOS-FLAG
actin


A
DS: 978
SS: 608
C
Soybean proteins
Soybean mRNAs
857
121
739
Dodder seeds
Dodder proteins
Dodder mRNAs
591
17
74
Soybean seeds
D
GUS
WT
500 μm
500 μm
B
Proteins
mRNAs
2.23%
1.37%
SS
97.77%
98.63%
ERPA (10^8)
CN
8.84%
0.41%
DS
91.16%
99.59%
Dodder
Soybean
E
Relative GUS activity
**
GUS
WT
F
Glyphosate
Water
***
Seeds germination rate/%
EPSPS
WT


A
SL: 949
AL: 791
B
8.80%
AL
91.20%
8.26%
SL
91.74%
ERPA (10^8)
15
10
5
0
Arabidopsis
Soybean
C
Soybean proteins in
Arabidopsis leaves
657
217
462
Arabidopsis proteins
in soybean leaves
D
Relative GUS activity
5000
4000
3000
2000
1000
0
***
***
eGFP-GUS
WT
Dodder stem
Soybean leaf
E


A
ERPA (10^8)
60
50
40
30
20
10
0
Arabidopsis (F)
Arabidopsis (O)
Soybean (F)
Soybean (O)
Dodder (F)
Dodder (O)
B
Intensities of mobile and non-mobile proteins
Frequency
1600
1200
800
400
0
non-mobile Arabidopsis
mobile Arabidopsis
non-mobile soybean
mobile soybean
non-mobile dodder
mobile dodder
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
11.5
12.0
Relative protein intensity (Log10 Intensity)
C
Frequency
12.00%
10.00%
8.00%
6.00%
4.00%
2.00%
0.00%
p=0.822128034
Dodder_F
Dodder_N
5
20
35
50
65
80
95
110
125
140
155
170
185
200
Molecular Weight (kDa)
p=5.64E-06
Arabidopsis_F
Arabidopsis_N
Molecular Weight (kDa)
D
Frequency
p=1.98E-15
Dodder_F
Dodder_N
Molecular Weight (kDa)
p=0.007092131
Soybean_F
Soybean_N
Molecular Weight (kDa)
E
Cell wall
2.5%
Extracell
0.7%
Cell membrane
5.0%
Undetermined
1.7%
Vacuole
2.0%
Golgi apparatus
2.0%
Endoplasmic
reticulum
2.0%
Peroxisome
2.5%
Mitochondrion
10.5%
Chloroplast
29.4%
Cytoplasm
15.7%
Nucleus
26.1%