Dodder-transmitted mobile signals prime host plants for enhanced salt tolerance

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Abstract

The dodders (Cuscuta spp.) are a genus of shoot parasites. In nature, a dodder often simultaneously parasitizes two or more neighboring hosts. Salt stress is a common abiotic stress for plants. It is unclear whether dodder transmits physiologically relevant salt stress-induced systemic signals among its hosts and whether these systemic signals affect the hosts’ tolerance to salt stress. Here, we simultaneously parasitized two or more cucumber plants with dodder. We found that salt treatment of one host highly primed the connected host, which showed strong decreases in the extent of leaf withering and cell death in response to subsequent salt stress. Transcriptomic analysis indicated that 24 h after salt treatment of one cucumber, the transcriptome of the other dodder-connected cucumber largely resembled that of the salt-treated one, indicating that inter-plant systemic signals primed these dodder-connected cucumbers at least partly through transcriptomic reconfiguration. Furthermore, salt treatment of one of the cucumbers induced physiological changes, including altered proline contents, stomatal conductance, and photosynthetic rates, in both of the dodder-connected cucumbers. This study reveals a role of dodder in mediating salt-induced inter-plant signaling among dodder-connected hosts and highlights the physiological function of these mobile signals in plant–plant interactions under salt stress.

Keywords: Dodder, inter-plant signaling, priming, salt stress, systemic signals, transcriptome.

Introduction

Plant growth and development are affected by adverse environmental factors, including herbivory, pathogen infection, extreme temperatures, drought, and salinity. Salt is very difficult to remove from farmland, posing a long-term stress that limits the growth and yield of all crops. Salt induces both osmotic stress and ion toxicity in plants (Parida and Das, 2005; Acosta-Motos et al., 2017), resulting in stomatal closure and consequent reductions in photosynthesis and transpiration (Chaves et al., 2009). Salt stress also induces secondary effects including nutritional disturbance, oxidative stress, membrane disorganization, and metabolic imbalance, and these physiological changes often result in inhibition of growth and even the death of plants (Xiong et al., 2002; Jin et al., 2016; Zhu, 2016). Pathways mediated by SALT OVERLY SENSITIVE (SOS) genes, calcium (Ca2+), reactive oxygen species (ROS), and the phytohormone abscisic acid (ABA) play important roles in mediating plant responses to ionic and osmotic stress (Evans et al., 2016; Yang and Guo, 2018).

Plant growth and development and plant adaption to environmental stresses require short- and long-distance intercellular,
inter-tissue, and inter-organ communications, which are mediated by different systemic signals. For example, leaves perceive day length, and, under flower-inducing conditions, such as short days for rice (*Oryza sativa*) and long days for Arabidopsis (*Arabidopsis thaliana*), the expression of the gene *flowering locus T* (*FT*) is induced; the systemic signal *FT* is then translocated to the shoot apex, where it triggers flowering (Turck et al., 2008; Song et al., 2015). Leaves are able to perceive insect feeding or pathogen infection and activate systemic defenses and systemic acquired resistance, respectively (Wu and Baldwin, 2010; Lu et al., 2013), enabling the leaves to enhance their defense against insects and pathogens before the insects move or the pathogens spread to them. As another example, in response to dehydration, a systemic signal, clavata3/embryo-surrounding region-related 25 (CLE25) peptide, is produced in the root and travels to the leaves to induce ABA biosynthesis, thereby reducing transpiration (Takahashi et al., 2018). Nitrate-induced root-shoot-root systemic signaling affects genome-wide transcriptome reprogramming and root development (Ruffel et al., 2011), and cytokinin was found to be involved in this systemic signaling in nitrogen-starved plants (Li et al., 2014).

**Priming** (previously called sensitization) is an important consequence of local and systemic responses (Conrath et al., 2015). After exposure to biotic or abiotic stresses, plants often prime themselves to respond faster and/or more strongly to the same or other stresses. For example, after being treated with necrotizing pathogens, salicylic acid, or many other natural or synthetic compounds, plants can enter a primed state in which they become more resistant to pathogens (Conrath et al., 2002). For example, wheat (*Triticum aestivum*) exhibited improved drought tolerance after initial drought priming (Wang et al., 2014). Accumulating evidence has revealed that plants can also be primed for salt tolerance after seeds or seedlings are treated with salt (Yan et al., 2015; Pandolfi et al., 2016; Ali et al., 2017; Berhane and Chala, 2017). Although the mechanisms underlying salt-induced priming remain unclear, compared with naive (non-primed) ones, primed plants show increased levels of organic solutes, such as proline (Pro) and sugars (Sivritpe et al., 2003), a higher photosynthetic rate (Yan et al., 2015), and increased abilities to sequester vascular sodium in the leaves and maintain low sodium content in the roots (Sivritpe et al., 2003; Nakaune et al., 2012; Yan et al., 2015; Pandolfi et al., 2016).

The genus *Cuscuta* (Convolvulaceae) comprises nearly 200 species, which are commonly named dodders. Dodders are stem holoparasites with no leaves or roots. Like all parasitic plants, dodders use a unique organ, the haustorium, to attach to and penetrate host stem tissue, forming xylem and phloem connections with the host's vasculature (Yoshida et al., 2016). Various molecules are able to move from host plants to dodders through haustorial junctions, including green fluorescent protein (Haupt et al., 2001), secondary metabolites (Birschwiks et al., 2006; Smith et al., 2016), mRNAs (Kim et al., 2014), small RNAs (Shahid et al., 2018), and even viruses (Birschwiks et al., 2006). Systemic signal transmission from dodder to host has also been demonstrated: aphids (*Myzus persicae*) feeding on the dodder *Cuscuta australis* induced systemic defense responses in host soybean (*Glycine max*), resulting in increased resistance of the host to insects (Zhuang et al., 2018).

Dodder stems often attach to multiple adjacent plants and establish parasitism on all of them, forming dodder–connected plant clusters composed of a dodder and two or more host plants. In these plant clusters, the dodder is very likely to be able to transfer various molecules between the hosts, including molecules with systemic signaling functions. For example, *C. australis* was found to convey *Spodoptera litura* caterpillar feeding-induced systemic signals from wounded soybean to the other hosts in the plant cluster, and induced a defense response in these plants (Hettenhausen et al., 2017).

Salt stress tolerance also involves systemic signaling. Arabidopsis seedlings have been found to propagate a salt-induced Ca2+ wave through the cortex and endodermal cell layers in a vacuolar ion channel TPC1-dependent manner, resulting in whole-plant salt stress tolerance (Choi et al., 2014); the Ca2+ wave requires AtRBOHD NADPH oxidase and TPC1 (Evans et al., 2016). Furthermore, treating alfalfa (*Medicago sativa*) root with salt induced systemic transcriptomic changes in leaves (Lei et al., 2018). In this study, we aimed to investigate whether dodder transmits salt stress-induced systemic signals among host plants and whether the inter-plant salt-induced systemic signals could have a priming effect on the neighboring dodder–connected host plants and enhance their salt tolerance.

**Materials and methods**

**Plant material and growth condition**

All plants were cultivated in a glasshouse with ~16 h light and 8 h dark, and temperature maintained at ~25°C (day) and ~18°C (night). Cucumber (*Cucumis sativus* cv. Junyou 35) seeds were germinated on moist filter paper, and after 5 days the seedlings were transferred to plastic cups with 2 litres of hydroponic culture solution (*MHS*). Plants were grown for a further 10 days, and then two or more cucumber plants in a row were placed next to each other (at ~15 cm distance edge to edge), allowing the dodders to parasitize the neighboring plants. After a further 15 days, the plant clusters were used for salt treatment experiments.

**Salt-induced priming experiments**

All salt solutions were prepared by dissolving NaCl in MHS. Mock treatment was done by refreshing the hydroponic solution with normal MHS. To prime unpasitized cucumber plants, individual plants were treated with 100 mM NaCl or mock treated (each group had 20 replicates); 3 days later, all the cucumber plants were supplied with MHS; after 3 days of resting, all the cucumber plants were treated with 70 mM NaCl for another 15 days. To study priming in plant clusters, in the pretreatment and control groups (each containing 20 replicated plant clusters), the first cucumber plant in each cluster was treated with 50 or 100 mM NaCl or (in controls) mock treated for 72 h; these plants were then supplied with normal MHS, and simultaneously, the dodder–connected neighboring cucumber plants were treated with 70 mM NaCl for 15 days. To study whether there is a salt-induced airborne signal, an experiment was performed as described for the experiment investigating priming in plant clusters, except that the cucumbers were infested with dodders but
no dodder connections between plants were allowed, and each pair of cucumbers was placed at a distance of ~15 cm (each group had 20 replicates). At the end of these experiments, the third leaf (counting from the oldest ones) of the cucumber plants were harvested.

**Analysis of salt-induced systemic signaling**

Plant clusters composed of two dodder-connected cucumber plants were used in experiments to investigate salt-induced systemic signaling. To study salt-induced early systemic signals, for each pair of hosts, one was mock treated (control group) or treated with 50 mM NaCl (treatment group); after 1 h, the other dodder-connected host in the pair was harvested. The control and treatment groups each consisted of six replicates. To determine transcriptomic and physiological changes in response to salt treatment, plant clusters were subjected to one of three treatments. In the control salt-treatment group, both hosts were treated with 50 mM NaCl; in the control non-salt-treatment group, both hosts were mock treated; in the treatment group, one host was treated with 50 mM NaCl and the other was mock treated. After 12, 24, and 72 h, samples were harvested from both hosts. Each group in this experiment had six replicates for each time point. At the end of these experiments, the third leaves (counting from the oldest ones) and the root tips (to a maximum of one-third of the total root) were harvested.

**RNA-seq and data analysis**

Total RNA was extracted from plant tissues using TRizol reagent (Thermo Fisher Scientific). Three biological replicates were used for each group of samples. Transcriptome sequencing was done to a depth (Thermo Fisher Scientific). Three biological replicates were used for RNA-seq and data analysis.

**Determination of stomatal conductance and photosynthetic rate**

Photosynthetic rate and stomatal conductance were measured using a LI-COR 6400 portable photosynthetic system (LI-COR Biosciences). The photosynthetic photon flux density was maintained at 1000 μmol m⁻² s⁻¹ and the concentration of CO₂ in the chamber was set to 400 μmol mol⁻¹. During the measurements, air relative humidity and leaf temperature were maintained at 60% and 25 °C, respectively.

**Analysis of free proline**

The free Pro content of each sample was determined following Bates et al. (1973) using 1-Pro (Sigma) as the standard. In brief, 50 mg of plant tissue was ground in liquid nitrogen and 1 ml of 3% (w/v) sulfosalicylic acid solution was added to each sample. After vortexing for 10 min, the solution was centrifuged at 5000 g for 20 min. Then, 0.5 ml of the supernatant was mixed with 1 ml of glacial acetic acid and acid ninhydrin (1:1, v/v) and kept in boiling water for 1 h. The reaction was stopped by placing the samples in a water bath at room temperature for 10 min, after which 1 ml of toluene was added to each reaction mixture and the samples were vortexed for 15 s. After centrifugation at 10 000 g for 5 min, the supernatants were transferred to fresh tubes. The absorption values of the supernatants were determined at 520 nm by using a spectrophotometer (Tecan).

**Evans blue assay**

The degree of leaf cell damage was analyzed using Evans blue staining following a previously published method (Baker and Mock, 1994). Cucumber leaf disks were excised and immediately immersed in a 0.25% (w/v) Evans blue solution for 24 h at room temperature. The leaf disks were rinsed extensively with water and then the chlorophyll was removed by boiling in an ethanol and glyc erin mixture (9:1, v/v) for 30 min. Images were taken with a camera (Canon). Subsequently, the leaf disks were immersed in a 1% sodium dodecyl sulfate solution for 3 days to extract the Evans blue. The absorbance values (at 600 nm) of the extracts were determined by using a spectrophotometer (Tecan).

**Extraction and quantification of abscisic acid**

Approximately 100 mg of plant tissue was ground in liquid nitrogen and 1 ml of ethyl acetate, spiked with 5 ng of the internal standard 2H₄-ABA (Olchemim), was added to each sample. After vortexing for 10 min, the samples were centrifuged at 13 000 g for 15 min at 4 °C. The supernatants were transferred to fresh tubes and evaporated to dryness in a vacuum concentrator (Eppendorf) at 30 °C. Then, 500 μl of 70% methanol (v/v) was added to each sample and the samples were vortexed for 10 min. After subsequent centrifugation at 13 000 g for 15 min at 4 °C, the supernatants were transferred to glass sample vials and loaded on to a UPLC-MS/MS system (LCMS-8040, Shimadzu), and quantification was conducted according to a previously published method (Wu et al., 2007).

**Determinant of chlorophyll content**

Fresh plant material (~100 mg per sample) was ground in liquid nitrogen and 1 ml of 80% (v/v) acetone was added to each sample. After vortexing for 10 min, the samples were centrifuged at 5000 g for 10 min. The supernatants were transferred to fresh tubes and their absorption values were determined at 646 and 663 nm by using a spectrophotometer (Tecan). The total chlorophyll contents of the samples were determined according to a previously published method (Arnon, 1949).
Determination of sodium contents

Samples were dried in an oven at 80 °C and homogenized by grinding. Approximately 0.1 g of the dried tissues was mixed with 7 ml concentrated nitric acid. The samples were then digested in a microwave oven (TOPLEX+, PreeKem). The clear digestion products were further concentrated to ~0.5 ml by heating at 170 °C and then diluted with water to 100 ml for determination of sodium contents using flame atomic absorption spectrometry (PinAAcle 900T, Perkin Elmer). Sodium contents were extrapolated from a standard curve constructed with serially diluted NaCl solutions.

Results

Systemic signal-induced priming effect in dodder-connected hosts

Cucumber (C. sativus) was chosen in this study as it is susceptible to dodder parasitization and can be easily cultured hydroponically, and the cucumber genome is available (Huang et al., 2009; Ling et al., 2017), facilitating RNA-seq analysis. Furthermore, the long-distance phloem-mobile signals of cucumber have been well studied (Lucas et al., 2013).

First, we examined whether salt treatment primes cucumber plants for increased salt tolerance. Cucumber plants were exposed to 100 mM NaCl or mock treated for 3 days, after which all the plants were supplied with normal hydroponic solution. After a further 3 days, all the cucumber plants were treated with 70 mM NaCl, and their phenotypes were assessed 15 days later. In the control group, all the plants showed severe withering, leaf chlorosis, and patchy necrosis, whereas the plants in the treatment group exhibited high tolerance to the salt treatment (Fig. 1A). Consistently, compared with the control group, plants in the treatment group showed 32% higher chlorophyll content (Fig. 1B). Leaf disks from the control group were heavily stained with Evans blue (Fig. 1C), while those from the treatment group were barely stained (Fig. 1C), and quantification of Evans blue showed 91% lower dye content in the leaves of the treatment group (Fig. 1D). These data indicate that salt pretreatment of cucumber plants can prime the plants for enhanced tolerance to subsequent salt stress.

To examine our hypothesis that treating one host in a dodder-connected plant cluster with salt may increase the salt tolerance of the other host(s), we created cucumber–dodder–cucumber plant clusters. The clusters were divided into three groups: control, treatment 1, and treatment 2 (Fig. 2A). For each pair of cucumber hosts connected by dodder, one cucumber plant was identified as P1 and the other as P2. In the control group, P1s were mock treated, while in the treatment 1 and 2 groups, P1s were treated with 50 or 100 mM NaCl, respectively. After 3 days, all P1s were supplied with normal hydroponic solutions, and P2s were treated with 70 mM NaCl for 15 days.

In the control group, the P2s displayed severe withering, leaf chlorosis, and patchy necrosis, whereas the P2s in treatment groups 1 and 2 all exhibited better tolerance to the salt treatment (Fig. 2A). The chlorophyll levels were similar in the P2s of the control and treatment 1 groups, but the P2s of the treatment 2 group had approximately 1.1-fold higher levels of chlorophyll (Fig. 2B). Next, we quantified relative electrolyte leakage values and cell death rates in the P2s. Salt stress-induced relative electrolyte leakage in the P2s of the control group was 26% and 44% greater than in the P2s of treatment groups 1 and 2, respectively (Fig. 2C). Consistent with the relative electrolyte leakage data, leaf disks from the P2s of the control group were heavily stained with Evans blue, while leaf disks from the P2s of treatment groups 1 and 2 showed less and much less staining, respectively (Fig. 2D); quantification of Evans blue in the disks indicated 68% and 91% lower dye content in the P2s of treatment groups 1 and 2 than in the control group (Fig. 2E), respectively. These results indicate that salt treatment of one of the hosts in a plant cluster activated systemic signals that moved to the other host via the dodder connection and primed the other host for salt tolerance.

To rule out the possibility that volatile compounds produced by plants in response to salt exposure function as signaling molecules to induce responses in adjacent plants, two cucumber plants infested with dodders were placed at the same distance as the dodder-connected cucumber hosts but dodders were not allowed to connect the adjacent hosts. In the treatment group, one plant in each host pair was treated with 50 mM NaCl, while in the control group, one plant in each host pair was mock treated. Three days later, all the adjacent hosts were treated with 70 mM NaCl for 15 days (Fig. 3A). After treatment, all the adjacent host plants were severely withered and showed leaf chlorosis and patchy necrosis (Fig. 3B), and leaf disks from these plants were all heavily stained by Evans blue (Fig. 3C, D). Thus, the possibility of salt-induced airborne signals between hosts can be ruled out.

Dodders often parasitize many adjacent host plants simultaneously. To study whether the inter-plant salt stress-induced systemic signals can travel to multiple hosts, we connected five cucumber plants in a row with dodders to form dodder-connected plant clusters (Fig. 4A). In the treatment group, the first plant (designated T1) was treated with 100 mM NaCl, and in the control group the first plant (designated C1) was mock treated; after 3 days all plants were supplied with normal hydroponic solution. Subsequently, the dodder-connected neighboring cucumber plants (T2–T5 and C2–C5 in the treatment and control groups, respectively) were treated with 70 mM NaCl for 15 days. In the control group, plants C2–C5 all exhibited severe withering and leaf necrosis. In contrast, plants T2–T4 showed only a weak salt stress phenotype, although T5 showed a moderate stress phenotype. The chlorophyll content, relative electrolyte leakage, and the content of Evans blue staining in the leaves of plants C2–C5 and T2–T5 were measured (Fig. 4). Compared with the plants at the respective positions in the control group, plants T2–T5 contained 2.1-, 2.8-, 2.3-, and 1.0-fold more chlorophyll (Fig. 4B). Furthermore, plants T2–T4 exhibited 26–15% less relative electrolyte leakage than their respective control plants, but the relative electrolyte leakage value of T5 was only 11% less than that of C5 (Fig. 4C). Leaf disks from these plants were stained with Evans blue (Fig. 4D). Quantification of Evans blue staining indicated that C2–C4 leaves contained 20- to 6-fold more Evans blue dye than T2–T4 leaves; the Evans blue content in the C5 leaves was approximately 1-fold more than in the T5 leaves (Fig. 4E).

These data indicate that the salt stress–induced systemic signals from one host plant can prime systemically connected host...
Dodder-transmitted mobile signals prime host plants for enhanced salt tolerance | 1175

Early responses induced by salt stress-activated systemic signals

After salt stress treatments, early transcriptomic changes in plants often play important roles in regulating the downstream defense/adaptation responses. Thus, we used transcriptome analysis to investigate the salt stress-induced early systemic signaling between dodder-connected pairs of hosts. In the control group, one host (C1) was mock treated, and in the treatment group one host (T1) was supplied with 50 mM NaCl (Fig 5A). After 1 h, 363 DEGs were identified between plants C2 and T2 (Fig. 5B), indicating that rapid systemic signals from salt-stressed T1 plants, presumably originating in the roots, were produced, traveled to the systemic T2 plants, and were perceived by their roots. To gain insight into the early regulated pathways in the roots of T2 plants, a GO analysis was performed on the DEGs between T2 and C2 roots; 'primary metabolic process', 'nitrogen compound metabolic process', and 'cellular macromolecule biosynthetic process' were among the enriched GO terms (Fig. 5B).

Notably, auxin, gibberellin, and ethylene signaling-related genes were found in the top 40 most regulated genes between C2 and T2 (Supplementary Fig. S1), implying that these phytohormones might be involved in signaling regulating the early systemic response in the roots of these plants. We chose four marker genes among the top 40 most regulated genes for qRT–PCR analysis: After 1 h salt treatment, in the T2 roots the transcript levels of genes encoding ethylene-responsive transcription factors (Csa4G652640 and Csa6G361330), dormancy/auxin-associated protein (Csa1G569400), and gibberellin 3-beta hydroxylase (Csa2G379300) were up- or down-regulated compared with the transcript levels in the C2 roots (Fig. 5C–F). These qRT–PCR results confirmed that salt stress-induced systemic signals can be transferred between hosts within 1 h. The speed of the salt-induced systemic signals, inferred from the distance between the roots of T1 and T2 plants (~70 cm), was estimated to be at least 1.2 cm min\(^{-1}\), since certain physiological responses, such as Ca\(^{2+}\) signaling, could be activated in the T2 plants earlier than 1 h.

Salt stress-induced transcriptomic reconfiguration in dodder-connected hosts

Next, we conducted a transcriptomic analysis to gain further insight into the physiological effect of dodder-transmitted systemic signals in host plants. Cucumber-dodder-cucumber plant clusters were divided into three groups. Two of these were control groups: first, the control salt treatment group (CS+), in which both hosts were treated with 50 mM NaCl, and second, the control non-salt-treated group (CS−), in which both hosts were mock treated. The third group was the treatment group, in which one host was treated with 50 mM NaCl (TS+), while the other was mock treated (TS−) (Fig. 6A). In this manner, pairwise comparisons allowed detailed inspection of the transcriptomic differences between the control groups and the treatment group.

After salt or mock treatment for 12 h and 24 h, the leaves and roots of the host cucumber plants were harvested for transcriptomic analysis. First, the numbers of DEGs were...
obtained from pairwise comparisons to reveal the transcriptomic differences between TS+ and TS−, and between CS+ and CS−. In leaves, at 12 h TS+ and TS− exhibited 952 DEGs, and there were 746 DEGs between CS+ and CS−; strikingly, at 24 h the differences between TS+ and TS− leaves almost completely disappeared (to 73), while the number of DEGs between CS+ and CS− leaves increased to 4426 (Fig. 6B). Similarly, in the root samples, at 12 h 349 and 387 DEGs were identified between TS+ and TS−, and between CS+ and CS−, respectively; at 24 h the number of DEGs between TS+ and TS− roots decreased to 168, while the DEGs between CS+ and CS− roots increased to 770 (Fig. 6C). Thus, in both leaf and root tissues,
the transcriptomic differences between CS+ and CS− became more pronounced over time, whereas the differences between the transcriptomes of TS+ and TS− greatly decreased.

Next, we compared TS− versus CS−, and TS+ versus CS+. At 12 h, the number of DEGs between TS− and CS− leaves was 317, and the number of DEGs between TS+ and CS+ leaves was 220; at 24 h, the number of DEGs between TS− and CS− leaves had increased to 1039, whereas there were 137 DEGs between leaves of TS+ and CS+ (Fig. 6B). The transcriptomic differences between TS− and CS− leaves indicate that certain mobile signals from the TS+ plants reached the TS− plants through the dodder bridges and altered their transcriptomes; similarly, the different transcriptomes of TS+ and CS− leaves also reveal that certain mobile feedback signals traveled from the TS− plants, which were not salt treated, to the TS+ plants, and altered the TS+ plants' transcriptomes. In the roots, 12 h after treatment, 245 DEGs were identified between the transcriptomes of TS− and CS−, whereas only 27 DEGs were found between TS+ and CS− roots; by 24 h, the number of DEGs between TS− and CS− roots increased to 584, but only 9 DEGs were found between TS+ and CS− roots (Fig. 6C). Consistent with the number of DEGs identified, Pearson correlation analysis based on FPKM values from all the DEGs also indicated the similarities among the CS+, TS+, and TS− transcriptomes at 24 h in both root and leaf samples (Fig. 6E, 6G); importantly, the similarity between the transcriptomes of TS− and TS+ leaves was greater than that between the transcriptomes of TS+ and CS+ leaves.

Given that the DEGs from the TS− and CS− transcriptomes reflect the regulation of the systemic signals from TS+ plants, we performed GO and pathway enrichment analysis on these DEGs to obtain insight into the physiological processes in the TS− plants that were regulated by systemic signals. Although many genes were expressed differentially in the leaves of TS− and CS− plants at both 12 and 24 h (317 and 1039, respectively), no biological processes or pathways were obviously enriched. However, the DEGs between TS− and CS− roots at 12 h showed enrichment in multiple processes (Supplementary Fig. S3), including ‘single-organism carbohydrate metabolic process’, ‘carbohydrate biosynthetic process’, and ‘oxidoreduction coenzyme metabolic process’ (Supplementary Fig. S3), and the significantly changed pathways included ‘glycolysis IV’ (Supplementary Fig. S3). When the salt treatment time was 24 h, processes enriched in the TS− roots compared with CS− roots included ‘single-organism metabolic process’, ‘oxidation-reduction process’, and ‘small molecule biosynthetic process’, and the significantly changed pathways included ‘Calvin–Benson–Bassham cycle’, ‘glycolysis IV’, and ‘sucrose biosynthesis I’.

The DEGs of CS+ and TS+ roots were very similar (Fig. 6C), but their leaf transcriptomes showed 220 and 137 DEGs at 12 and 24 h, respectively (Fig. 6B). Even though GO analysis
on these DEGs resulted in no enrichment, we found that these DEGs in leaves included several genes involved in carbohydrate metabolism and Ca\textsuperscript{2+} and phytohormone (auxin, gibberellin) signaling (Supplementary Fig. S4).

In addition, we specifically inspected the relative transcript levels of DEGs (69 and 24 DEGs in leaves and roots, respectively, at 12 h, and 111 and 48 DEGs in leaves and roots, respectively at 24 h) that are known to be involved in salt tolerance, including those related to second messenger signaling (ROS and Ca\textsuperscript{2+}), phytohormone signaling (auxin, abscisic acid, jasmonic acid, gibberellin, ethylene), and ion transport (Park et al., 2016; Zhu, 2016). Heatmap and hierarchical clustering analysis on these genes again indicated relatively high similarities among these genes’ transcript profiles in the leaves and roots of TS–, TS+, and CS+ plants at 24 h (Supplementary Fig. S5).

Together, these transcriptome data indicate that dodders transferred salt-induced systemic signals from TS+ plants to TS– plants. Importantly, the systemic signals transferred from TS+ to TS– plants induced large transcriptomic changes in the TS– plants, and by 24 h the transcriptomes of the salt-treated CS+ and TS+ plants and the non-salt-treated TS– plants were remodeled to a similar profile. These transcriptomic changes in the TS– plants may have contributed at least partly to their highly increased salt tolerance to the subsequent salt treatment (i.e. a priming effect).

Salt stress-induced physiological responses in dodder-connected hosts

Plants often respond to salt stress with increased Pro and ABA content, increased oxidase activity, and reduced photosynthesis due to closure of stomata (Zhu, 2003; Chaves et al., 2009). To gain insight into the mechanism by which dodder-transmitted mobile signals prime host plants for enhanced salt tolerance, we established cucumber–dodder–cucumber plant clusters as illustrated in Fig. 6A.

At 12, 24, and 72 h after treatment, photosynthetic rates and stomatal conductance values, and the contents of Pro and ABA were quantified in the CS+, CS–, TS+, and TS– plants. At 12 h, the photosynthetic rate of TS+ plants was 32% lower than that of CS+ plants, while CS+, CS–, and TS– exhibited similar photosynthetic rates (Fig. 7A). At 24 h, the photosynthetic rate of CS+ decreased by 24%, but the photosynthetic rates of all the other plants showed no obvious change (Fig. 7A). At 72 h, the photosynthetic rates of TS+ and CS+ plants further decreased to a similar level, but the photosynthetic rate of TS– plants was 36% higher than that of the CS– plants.
Fig. 5. Salt treatment rapidly activates dodder-mediated inter-plant systemic signaling. (A) Schematic of the experiment setup. Twelve plant clusters were divided into two groups. In the control group, one host in each cluster was mock treated (C1) and the other host was untreated (C2); in the treatment group, one host in each cluster was treated with 50 mM NaCl (T1) and the other host was untreated (T2). Root samples of C2 and T2 plants were harvested 1 h after treatment. (B) Number of DEGs from the comparison between the T2 and C2 root transcriptomes and the enriched GO terms of these DEGs. Detailed transcriptome information is shown in Supplementary Table S3 (n=3; each replicate was pooled from two biological replicates). (C–F) qRT–PCR analysis of the transcript levels of genes encoding ethylene-responsive transcription factor (Csa4G652640) (C), ethylene-responsive transcription factor (Csa6G361330) (D), dormancy/auxin-associated protein (Csa1G569400) (E), and gibberellin 3-beta hydroxylase (Csa2G379300) (F), which were selected from the top 40 most regulated genes in the T2 roots. Data are means ±SE (n=6). Asterisks indicate significant differences between the C2 and T2 plants: *P<0.05, **P<0.01 (Student’s t-test). The heatmaps indicating the transcript levels of the same four genes from RNA-seq analysis are highlighted with red frames in Supplementary Fig. S1A.

(Fig. 7A) The TS– plants showed similar stomatal conductance to the CS– plants at 12 and 24 h, and at 72 h the stomatal conductance of the TS– plants was ~40% higher than that of the CS– plants (Fig. 7B); the TS+ and CS+ plants showed largely similar stomatal conductance, which was ~50% lower than that in the TS– and CS– plants (Fig. 7B).
Fig. 6. Salt stress induces large transcriptomic reconfigurations in dodder-connected hosts. (A) Schematic of cucumber–dodder–cucumber plant clusters and salt treatment. CS+, both hosts in the pair were treated with salt (50 mM NaCl was used for all salt treatments in this experiment); TS+, one host in the pair was treated with salt; TS−, the other host in the pair was mock treated; CS−, both hosts in the pair were mock treated. Leaf and root samples of CS+, CS−, TS+, and TS− plants were harvested 12 and 24 h after treatment for RNA-seq analysis. (B, C) Numbers of DEGs from TS+ versus TS−, CS+ versus CS−, TS− versus CS−, and TS+ versus CS+ of leaves (B) and roots (C) after salt treatment for 12 h and 24 h. (D–G) Pearson correlation coefficient heatmaps of all DEGs from TS+, CS+, TS−, and CS− leaves (D, E) and roots (F, G) after salt treatment for 12 h and 24 h. The value shown in each cell corresponds to the Pearson correlation coefficient for that pairwise comparison. Detailed information on the DEGs is shown in Supplementary Table S3 (n=3; each replicate was pooled from two biological replicates).
Pro content did not show any differences between any leaf samples at 12 and 24 h (Fig. 7C); at 72 h the Pro content of the CS+, TS+, and TS– leaves increased 39%, 60%, and 43%, respectively, while no change was detected in CS– leaves (Fig. 7C). In CS+ roots, the Pro content was always approximately 3-fold greater than in the CS– roots (Fig. 7D); compared with the CS+ roots, TS+ roots had similar levels of Pro at 12 and 24 h, but at 72 h Pro content in the TS+ roots increased to be 70% higher than in the CS+ roots (Fig. 7D). While TS–, CS+, and CS– plants exhibited similar leaf ABA contents, the TS+ leaves had approximately 1-fold more ABA than the leaves of the other plants (Fig. 7E). In CS– and TS– roots, ABA contents were similar at all times (Fig. 7F); although the ABA contents in the CS+ and TS+ roots at 12 h were similar to those in the TS– and CS– roots, at 24 and 72 h the CS+ and TS+ roots both exhibited approximately 2-fold increased ABA contents (Fig. 7F).

These data suggest that although the TS– plants were not salt treated, certain systemic signals from the TS+ plants traveled through the dodder to the TS– plants and induced physiological responses in them, and the increase of leaf and root Pro in the TS– plants, which was induced by the dodder-mediated systemic signals, may have partly contributed to salt tolerance in the TS– plants.

Finally, to determine whether salt can be translocated from one host to another through dodder, we determined the sodium contents in the leaves and roots of CS+, TS+, TS–, and CS– cucumber plants harvested 24 and 72 h after treatment (CS– plants were the controls) (Fig. 6A). In leaves, TS– plants did not show any changes in sodium content at either of the times examined, while in the TS+ plants, sodium concentrations were 77% and 2.2-fold higher, respectively, at 24 and 72 h; similarly, the CS+ plants exhibited 50% and 1.4-fold higher sodium content at 24 and 72 h (Supplementary Fig. S6A). The sodium contents in TS– and CS– roots were similar at both times examined (Supplementary Fig. S6B). Remarkably, the sodium contents in TS+ and CS+ roots were 10– and 10.7-fold higher, respectively, at 24 h, and at 72 h even greater levels of sodium were detected in TS+ and CS+ roots (Supplementary Fig. S6B). Thus, after salt treatment, the roots of CS+ and TS+ plants contained high levels of NaCl; however, the sodium contents of the leaves of these plants increased only slightly. Importantly, no detectable amount of sodium was transferred to the TS– plants through the dodder connection, providing evidence against the suggestion that salt itself is the systemic signal that induced large transcriptomic reconfiguration in the TS– plants.

Fig. 7. Salt stress induces physiological alterations in dodder-connected hosts. The experimental setup is shown in Fig. 6A. Photosynthetic activity (A), stomatal conductance (B), content of proline in leaves (C) and roots (D), and concentration of ABA in leaves (E) and roots (F) were determined in CS+, CS–, TS+, and TS– cucumber plants harvested 12 and 24 h after treatment. Data are means ± SE (n=6). Bars with the same letters are not significantly different from each other (P>0.05; one-way ANOVA and Duncan’s multiple range test).
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In nature, dodder can parasite multiple hosts simultaneously and can form vascular connections through haustoria. Other than water and nutrients, it remains poorly understood which molecules, including those with signaling functions (systemic signals), can be transferred between host and dodder, and between hosts via dodder. Although salt contamination usually occurs in relatively large areas of land, and thus affects whole communities of plants simultaneously, the dodder–host system can be used as a new tool to dissect salt-induced systemic signaling. In this study, we demonstrated that dodder transmits salinity stress-induced systemic signals between dodder-connected cucumber hosts and, importantly, these inter-plant systemic signals have a priming effect against salt stress on the neighboring hosts.

Besides the treatment group (TS+~dodder~TS~), we included two control groups (CS+~dodder~CS+ and CS~~dodder~CS~) in our experiments (Fig. 6A). This setup enabled us to make pairwise comparisons to gain detailed insight into the effect of the mobile signals on the plant transcriptome and other salt stress–related physiological changes. Moreover, our transcriptome analysis also provides some evidence of the mechanism by which the inter-plant mobile signals primed the host plants for salt tolerance. Dodder–transmitted systemic salt stress signals induced large transcriptomic reconfigurations in the non-salt–treated TS~ hosts to a level similar to the transcriptomes of salt–treated CS+ and TS+ plants (Fig. 6), including a number of genes involved in ROS, Ca2+, ion transport, and phytohormone signaling (Supplementary Fig. S5). These transcriptomic reconfigurations in the TS~ plants very likely changed these plants’ physiology to a salt–tolerant state, as supported by the increased Pro contents in the TS~ leaves and roots (Fig. 7C, D). However, in the absence of exposure to salt stress, these TS~ plants maintained normal photosynthesis and stomatal conductance (Fig. 7A, B), probably providing the TS~ plants with normal energy and resources; in contrast, CS+ and TS+ plants had much lower photosynthesis and stomatal conductance. All these factors are at least part of the mechanism for the priming of salt tolerance in the TS~ plants.

Intriguingly, carbohydrate–related processes were enriched in the DEGs between TS~ and CS~ roots (Supplementary Fig. S3). Thus, sugar metabolism or signaling might be implicated in salt tolerance priming in the TS~ host plants. This is consistent with the findings that the fructan content was positively correlated with the degree of salt tolerance in different wheat genotypes (Kerepesi and Galiba, 2000) and, in the desert soil alga *Microcoleus vaginatus*, the sodium concentration of salt-stressed cells decreased markedly after supplementation of sucrose (Chen et al., 2006).

The nature of the systemic signals that travel from TS+ to TS~ plants is unclear. However, our analysis indicated that they are rapidly moving (more than 1.2 cm min⁻¹) and located upstream of the regulatory networks, as they are able to change various aspects of TS~ physiology, including dramatic remodeling of the transcriptome, and finally prime the other connected hosts against salt stress. These systemic signals are probably produced in the roots, which are directly exposed to salt, then transmitted to the shoots of the host plant and thereafter translocated via dodder to the other hosts, where they activate stress–related responses. It is plausible that these systemic signals are part of an intricate systemic signaling network that allows different parts of a plant to communicate, so as to maintain the best physiological output for adapting to salt stress. In a dodder–connected plant cluster, salt stress–induced systemic signals not only travel from the host roots to shoot but, through vascular fusion, they are also disseminated first to the dodder and then to the other hosts in the cluster by an apoplastic and/or symplastic transfer mechanism shared by the dodder and the host plants. Given that these systemic signals can be transferred between cucumber (*Cucurbitaceae*) and dodder (*Convolvulaceae*) and induce physiological changes, it is likely that these signals are conserved in eudicots.

It has previously been demonstrated that herbivory–induced systemic signals alert the hosts in a dodder–connected plant cluster and lead to an elevation of their defenses against insects (Hettenhausen et al., 2017). We show that, in addition to herbivory–induced systemic signals, the dodder–mediated inter–plant systemic signals can convey physiologically meaningful information between the hosts within a plant cluster, enhancing their tolerance to salt stress. It is very likely that dodder plays a broad role in transferring various stress–induced systemic signals among the hosts in a plant cluster and thus alters how these host plants interact with or adapt to environmental factors.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. The recipe for modified Hoagland solution.
Table S2. List of primers used for RT–qPCR analysis.
Table S3. Differentially regulated genes from TS+ versus TS~, CS+ versus CS~, TS~ versus CS~, and TS+ versus CS+.

Fig. S1. The top 40 most regulated genes between T2 and C2 roots.

Fig. S2. Venn diagram analysis of the transcriptomic responses to salt stress in dodder–connected hosts.

Fig. S3. Transcriptomic comparison between TS~ and CS~ plant roots.

Fig. S4. Heatmap representing the expression of DEGs obtained between TS+ and CS+ leaves, which are involved in salt stress signaling.

Fig. S5. Heatmap representing relative transcript abundance of DEGs that are known to be involved in salt stress adaptation.

Fig. S6. Sodium concentrations in dodder–connected CS+, CS~, TS+, and TS~ host plants.

Data availability

All the transcriptome data can be accessed at the NCBI (https://ncbi.nlm.nih.gov) under BioProject ID PRJNA524275.
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References

Acosta-Motos J, Ortuño M, Bernal-Vicente A, Díaz-Vivancos P, Sanchez-Blanco M, Hernandez J. 2017. Plant responses to salt stress: adaptive mechanisms. Agronomy 7, 2–8.

Ali Q, Daud MK, Haider MZ, et al. 2017. Seed priming by sodium nitroprusside improves salt tolerance in wheat (Triticum aestivum L.) by enhancing physiological and biochemical parameters. Plant Physiology and Biochemistry 119, 50–58.

Anders S, Huber W. 2010. Differential expression analysis for sequence count data. Genome Biology 11, R106.

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology 24, 1–15.

Baker CJ, Mock NM. 1994. An improved method for monitoring cell death in cell suspension and leaf disc assays using Evans blue. Plant Cell Tissue & Organ Culture 39, 7–12.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant & Soil 39, 205–207.

Becker RA, Chambers JM, Wilks AR. 1985. The new S language: a programming environment for data analysis and graphics. The Economic Journal 100, 650–658.

Berhane GG, Chala AQ. 2006. Effects of salt priming in maize (Zea mays L.) leaf. Ethiopian Journal of Biological Sciences 16, 209–223.

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.

Bolger AM, Lohse M, Usadel B. 2014. Trimomatic: a flexible trimmer for illumina sequence data. Bioinformatics 30, 2141–2142.

Chaves MM, Flexas J, Pinheiro C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of Botany 103, 551–560.

Chen LZ, Li DH, Song LR, Hu CX, Wang GH, Liu YD. 2006. Effects of salt stress on carbohydrate metabolism in desert soil alga Cystoseira australis. Journal of Applied Phycolgy 18, 97–119.

Choi WG, Gilroy S, Morris RJ. 2015. A ROS-assisted calcium wave dependent on the AtRBOHD NADPH oxidase and TPC1 cation channel propagates the systemic response to salt stress. Plant Physiology 171, 1771–1784.

Haas BJ, Papanicolaou A, Yassour M, et al. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nature Protocols 8, 1494–1512.

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.

Hettenhausen C, Li J, Zhuang H, et al. 2017. Stem parasitic plant Cuscuta australis (dodder) transfers herbivory-induced signals among plants. Proceedings of the National Academy of Sciences, USA 114, E6703–E6709.

Hoagland DR, Arnon DI. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Extension Service 347, 357–359.

Huang S, Li R, Zhang Z, et al. 2009. The genome of the cucumber, Cucumis sativus L. Nature Genetics 41, 1275–1281.

Jin PH, Woo-Yeon K, Yun DJ. 2016. A new insight of salt stress signaling in plant. Molecules & Cells 39, 447–459.

Keresepi I, Galiba Gb. 2000. Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. Crop Science 40, 482–487.

Kim G, LeBlanc ML, Wafulla EK, dePamphilis CW, Westwood JH. 2014. Genomic-scale exchange of mRNA between a parasitic plant and its hosts. Science 345, 808–811.

Lei Y, Xu Y, Hettenhausen C, Lu C, Shen G, Zhang C, Li J, Song J, Lin H, Wu J. 2018. Comparative analysis of alfalfa (Medicago sativa L) leaf transcriptomes reveals genotype-specific salt tolerance mechanisms. BMC Plant Biology 18, 35.

Li J, Hettenhausen C, Sun G, Zhuang H, Li JH, 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.

Li Y, Krouk G, Coruzzi GM, Ruffel S. 2014. Finding a nitrogen niche: a systems integration of local and systemic nitrogen signalling in plants. Journal of Experimental Botany 65, 5601–5610.

Ling J, Luo Z, Liu F, Mao Z, Yang Y, Xie B. 2017. Genome-wide analysis of microRNA targeting impacted by SNPs in cucumber genome. BMC Genomics 18, 275.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15, 550.

Liu BB, Li XJ, Sun WW, et al. 2013. AtMYB44 regulates resistance to the green peach aphid and diamondback moth by activating EIN2-affected defences in Arabidopsis. Plant Biology 15, 841–850.

Lucas WJ, Groover A, Lichtenberger R, et al. 2013. The plant vascular system: evolution, development and functions. Journal of Integrative Plant Biology 55, 294–388.

Nakane M, Hanada A, Yin YG, Matsukura C, Yamaguchi S, Ezura H. 2012. Molecular and physiological dissection of enhanced seed germination using short-term low-concentration salt seed priming in tomato. Plant Physiology and Biochemistry 52, 28–37.

Pandolfi C, Azzarello E, Mancuso S, Shabala S. 2016. Acclimation improves salt stress tolerance in Zea mays plants. Journal of Plant Physiology 201, 1–8.

Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60, 324–349.

Park HJ, Kim WY, Yun DJ. 2016. A new insight of salt stress signaling in plant. Molecules and Cells 39, 447–459.

Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM. 2011. Nitrogen economics of root foraging: transitive closure of the nitrate–cytokinin relay and distinct systemic signaling for N supply vs. demand. Proceedings of the National Academy of Sciences, USA 108, 18524–18529.

Shahid S, Kim G, Johnson NR, et al. 2018. MicroRNAs from the parasitic plant Cuscuta campestris target host messenger RNAs. Nature 553, 82–85.

Srivitepe N, Srivitepe HO, Eris A. 2003. The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. Scientia Horticulturae 97, 229–237.

Smith JD, Woldemariam MG, Mescher MC, Jander G, De Moraes CM. 2016. Glucosinolates from host plants influence growth of the parasitic plant Cuscuta gronovii and its susceptibility to aphid feeding. Plant Physiology 172, 181–197.

Song YH, Shin JS, Kinmonth-Schultz HA, Imaiizu T. 2015. Photoperiodic flowering: time measurement mechanisms in leaves. Annual Review of Plant Biology 66, 441–464.

Takahashi F, Suzuki T, Osakabe Y, Betsuyaku S, Kondo Y, Dohmae N, et al. 2014. Genomic-scale exchange of mRNA between a parasitic plant and its hosts. Science 345, 808–811.

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Turck F, Fornara F, Coupland G. 2008. Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annual Review of Plant Biology 59, 573–594.

Wang X, Vignjevic M, Jiang D, Jacobsen S, Wollenweber B. 2014. Improved tolerance to drought stress after anthesis due to priming before anthesis in wheat (Triticum aestivum L.) var. Vinjett. Journal of Experimental Botany 65, 6441–6456.

Wu J, Baldwin IT. 2010. New insights into plant responses to the attack from insect herbivores. Annual Review of Genetics 44, 1–24.

Wu J, Hettenhausen C, Meldau S, Baldwin IT. 2007. Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of Nicotiana attenuata. The Plant Cell 19, 1096–1122.

Xiong L, Schumaker KS, Zhu JK. 2002. Cell signaling during cold, drought, and salt stress. The Plant Cell 14 (Suppl 1), S165–S183.

Yan K, Xu H, Cao W, Chen X. 2015. Salt priming improved salt tolerance in sweet sorghum by enhancing osmotic resistance and reducing root Na⁺ uptake. Acta Physiologiae Plantarum 37, 37–203.

Yang Y, Guo Y. 2018. Unraveling salt stress signaling in plants. Journal of Integrative Plant Biology 60, 796–804.

Yoshida S, Cui S, Ichihashi Y, Shirasu K. 2016. The haustorium, a specialized invasive organ in parasitic plants. Annual Review of Plant Biology 67, 643–667.

Zheng Y, Wu S, Bai Y, et al. 2019. Cucurbit Genomics Database (CuGenDB): a central portal for comparative and functional genomics of cucurbit crops. Nucleic Acids Research 47, D1128–D1136.

Zhu JK. 2003. Regulation of ion homeostasis under salt stress. Current Opinion in Plant Biology 6, 441–445.

Zhu JK. 2016. Abiotic stress signaling and responses in plants. Cell 167, 313–324.

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