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Effect of environmental conditions on seed germination and seedling growth in *Cuscuta campestris*

Koki Nagao[1] · **Taku Takahashi**[1] · **Ryusuke Yokoyama**[2]

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Abstract
Dodder (*Cuscuta*) is an obligate parasitic plant that cannot survive without a host and causes significant damage to crop yields. To understand its growth characteristics before parasitism, we examined the effects of environmental conditions on seed germination and seedling growth in *Cuscuta campestris* Yunck. Among various factors, we focused on the effects of light, pH, temperature, sugars, salts, hormones, amino acids and polyamines on seeds sown on agar plates. Regarding the effect of light on germination, far-red light was preferable rather than red light and the reversible response of seeds to red and far-red light was confirmed, implicating a phytochrome-mediated signaling pathway opposite to that in many seed plants. Among the amino acids, aspartic acid and alanine had a promotive effect, while histidine had an inhibitory effect on germination. We further found that, in addition to gibberellic acid, methyl jasmonate stimulated both germination and shoot elongation. While 2,4-D extended the viability of trichomes around the root cap, kinetin induced the formation of scale leaves on the shoot and undifferentiated cell clusters at the base of the shoot and root tip. Real-time reverse transcriptase PCR (RT-PCR) experiments confirmed that the expression of a putative *RbcS* gene for photosynthesis showed no response to light, whereas that of a *Phytochrome A* homolog increased in the dark. Our results indicate that some of the molecular mechanisms involved in responding to light and hormone signals are uniquely modified in dodder seedlings, providing clues for understanding the survival strategy of parasitic plants.

Keywords *Cuscuta* · Environmental conditions · Germination · Hormone responses · Seedling growth

Introduction

Cuscuta species (dodders) are among the most widespread parasitic weeds, attaching to and feeding on the stems of various herbaceous angiosperms. Understanding the physiological traits of dodders is critical for developing effective pest control systems, as they cause significant damage to many important crops. There is an increasing number of studies on the development of the haustorium, a root-like structure that penetrates the host's tissue and draws water and nutrients (Yoshida et al. 2016; Shimizu and Aoki 2019; Jhu and Sinha 2022; Hartenstein et al. 2023). Blue and far-red light strongly enhance the process of parasitism. A low ratio of red to far-red wavelengths (R/FR) slows down the circumnutation of dodder seedlings (Johnson et al. 2016) but significantly promotes entanglement and haustorium formation (Pan et al. 2022). The elongation of the haustorium is promoted by host-produced ethylene (Narukawa et al. 2021). The involvement of ethylene in haustorium formation has also been shown in a facultative root parasite in the Orobanchaceae family, *Phtheirospermum japonicum* (Cui et al. 2020). Furthermore, as-yet-unidentified host-derived signaling factors may be important for the establishment of parasitic connection between dodders and host plants.

In contrast to the host-dependent growth during parasitism, growth before parasitism mainly depends on the starch stored in the seedlings as an energy source. However, the developmental control and physiological traits of dodder seedlings during this growth phase remain largely unexplored. They emerge with a thread-shaped hypocotyl and

✉ Taku Takahashi
perfect@cc.okayama-u.ac.jp

✉ Ryusuke Yokoyama
ryusuke.yokoyama.d6@tohoku.ac.jp

[1] Graduate School of Environmental, Life, Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

[2] Graduate School of Life Sciences, Tohoku University, Sendai 980-0812, Japan

no cotyledons, which exhibits nastic movements in search of potential hosts. Most dodders form only rudimentary roots with root apices surrounded by a circle of root hair-like trichomes. The shoots have an apical meristem but lack mechanical tissues (Toma et al. 2005; Sherman et al. 2008). Xylem bundles show scattered or circular arrangement, depending on species (Toma et al. 2005). The seedlings become senescent within 7 to 10 days and collapse completely within 2 weeks post-germination. It is thus essential for their survival to locate host plants during this short period. In addition, the characteristics of seed dormancy, germination, and dispersal methods are crucial for the success of plant species. For parasitic plant seeds, germination must be triggered by the coincidence of specific environmental conditions for successful seedling establishment, along with the nearby growth of a host plant. In an experiment on eastern dodder (*Cuscuta monogyna* Vahl.), seed germination was found to be severely affected by soil depth. However, when seeds were after-ripened at different depths in the field, the highest germination occurred at a 5 cm soil depth, 270 days after burial (Ebrahimi et al. 2022). A study revealed that *Cuscuta australis* R.Br. seeds and seedlings are highly insensitive to abscisic acid (ABA), suggesting that the ABA-mediated drought resistance pathway in dodders may have degenerated during evolution (Li et al. 2015). In contrast, extracts from several herbaceous plants, including *Inula viscosa*, *Dracocephalum moldavica*, *Nepeta meyeri*, and *Caccinia macranthera*, have been shown to inhibit dodder germination (Andolfi et al. 2013; Movassaghi and Hassannejad 2015; Shekari et al. 2022; Ghanbari et al. 2023). Natural metabolites derived from fungi or microorganisms could be utilized in parasitic weed management strategies, such as preventing host attachment and stimulating germination in the absence of the host (Vurro et al. 2009).

In the current study, to understand the characteristics of dodders prior to parasitism, we focus on the responses of *C. campestris* seeds and seedlings to a variety of external conditions. Our results provide a clue for further exploring the survival strategy of dodders and the pest control against them.

Materials and methods

Plant materials and growth conditions

Seeds of *C. campestris* (Narukawa et al. 2021) were harvested from the plants parasitizing *Arabidopsis thaliana* (L.) Heynh., the Columbia-0 ecotype, grown under a 16/8 h light/dark cycle at 25 °C. Dry *C. campestris* seeds were soaked in concentrated sulfuric acid for 25 min, rinsed three times with distilled water, sterilized in bleach solution containing 1% sodium hypochlorite for 10 min, and washed five times with distilled water. The seeds were then sown on 1% agar plates containing 1x Murashige and Skoog (MS) salts and 1% (w/v) sucrose and incubated under a 16/8 h light/dark cycle of fluorescent white light (30 µmol m^{-2} s^{-1}) at 25 °C for 3 days, unless otherwise stated. In the seedling growth experiments, germinating seedlings that showed full emergence of the root on day 3 after sowing were transferred to new plates and allowed to grow for 7 days under different conditions. The conditions tested were light, pH, temperature, salinity, sugars, hormones, amino acids, and polyamines.

For light treatments, plates were illuminated with approximately 7.5 µmol m^{-2} s^{-1} of blue light (λ_{max} = 450 nm, Tokulumi Company Limited, China), red light (λ_{max} = 660 nm, Tokulumi Company Limited), or 5 µmol m^{-2} s^{-1} of far-red light (λ_{max} = 720 nm, Fuji electronic, Japan). For pH experiments, we tested 7 different pH regimes: 5.0, 6.0, 7.0, 7.5, 8.0, 8.5, and 9.0. The MS media, which was supplemented with 10 mM tris base, was adjusted to the desired pH with 1 N HCl. For temperature treatments, 4, 15, 20, 25, 30, and 35 °C were examined and plates were placed agar-side down to reduce the effect of humidity changes on the growth. For sugar treatments, 100 mM sucrose, glucose, maltose, and mannose were tested. All 20 amino acid treatments were examined at equimolar concentration (1 mM). Polyamines tested were 1 mM putrescine, 1 mM spermidine, 100 µM spermine, and 100 µM thermospermine.

Microscopy

Seedlings were imaged with a stereomicroscope (SMZ1500, Nikon, Japan) and the shoot length was analyzed with the ImageJ software. For tissue sections, samples were fixed overnight in 50% ethanol, 5% formaldehyde and 5% acetic acid, dehydrated through an ethanol series from 50% with water (v/v) to 100%, and embedded in Technovit 7100 resin (Heraeus Kulzer, Germany) according to the manufacturer's instructions. Sections of 10 µm thickness were stained with 0.1% toluidine blue and observed under a light microscope (ECLIPS 80i, Nikon).

RNA extraction and qRT-PCR

For Total RNA was prepared from seedlings using a NucleoSpin RNA Plant Kit (Takara, Japan) according to the manufacturer's instructions and reverse-transcribed using a PrimeScript RT reagent Kit (Takara) with the oligo-dT primer. The resulting first-strand cDNA was used as a template for real-time PCR with gene-specific primers. PCRs were performed using a KAPA SYBR FAST qPCR Kit (KAPA Biosystems, USA) with the Thermal Cycler Dice

Real Time System (Takara). Thermal cycling conditions were as follows: initial denaturation at 94℃ for 5 min; 35 cycles at 94℃ for 30 s, 55℃ for 30 s, and 72℃ for 30 s. *EF1α* was used as an internal standard. Specific primers used for each cDNA amplification are shown in Fig. S1.

Chemicals

MS salts, sugars, amino acids, and polyamines except thermospermine were purchased from Fujifilm Wako (Japan). Thermospermine was purchased from Santa Cruz Biotechnology (USA). All plant hormones and bikinin were obtained from Tokyo Chemical Industry (Japan).

Statistical analysis

Three replicates of 20 seeds for germination and 10 seedlings for shoot growth were examined in each treatment. The data were analyzed by Student's *t*-test between control and experimental groups or oneway ANOVA with Tukey–Kramer multiple comparison test.

Results

Far-red light enhances seed germination

Seeds were treated with concentrated sulfuric acid and then sown on MS agar plates. Under our experimental conditions, the germination rate nearly reached its maximum within 6 days after sowing (Fig. 1A). In this study, the seeds were judged as germinated based on visible root protrusion (Fig. 1B). The germinating seedlings were transferred 3 days after sowing to new agar plates and grown under a 16/8 h light/dark cycle of fluorescent light. The shoot length reached its maximum at 10 days after sowing (7 days after transfer; Fig. 1C). Figure 1D shows the typical morphology of 10-day-old seedlings.

We first examined the effect of light conditions on seed germination and shoot growth. When the plates were exposed to continuous white, red, far-red, or blue light, or darkness for 3 days after sowing, germination was enhanced under far-red, blue, and darkness conditions, but reduced under red light compared to white light (Fig. 2A). We then examined the effect of alternating 24-h incubation periods with red and far-red light and found that the light applied later was more effective for germination (Fig. 2B), suggesting the involvement of reversible phytochrome regulation. After transferring 3-day-old seedlings that had germinated under white light, shoot growth was examined under different light conditions. Haustorium formation was confirmed to be induced by far-red light (Fig. 2C), while shoot length was shortened under far-red light compared to other light conditions (Fig. 2D).

Alkaline pH limits seed germination

When the seeds were placed on agar media with different pH conditions, germination was significantly reduced at pH above 8.0 (Fig. 3A). Shoot elongation was also reduced at pH higher than 8.0 but not affected by the acidic pH of 5.0 (Fig. 3B). Regarding temperature, the results showed that high temperatures up to 30 °C promoted germination while low temperatures below 20 °C were inhibitory (Fig. 3C). This confirms a previous study proposing a model for temperature-dependent germination of *C. campestris* seeds (Goldwasser et al. 2016). Similar responses to temperatures were observed for shoot growth (Fig. 3D). We also examined the effect of sugars and high salts on germination. Among various sugars, sucrose, glucose, maltose (a major product of starch breakdown), and mannose (which could be provided from plant cell walls) were tested. However,

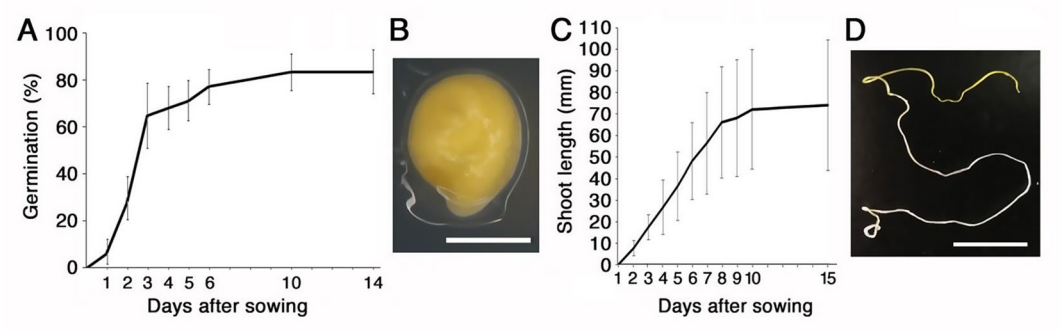

Fig. 1 The rate of germination and shoot growth of *C. campestris*. (**A**) Time course of the germination rate of the seeds, which were sown on MS agar plates and placed under 16/8 h white light/dark cycle at 25 °C. Data are means ± SE ($n = 3$, 20 seeds each). (**B**) Morphology of the seed that begins root protrusion and is judged germinated. Bar = 1 mm. (**C**) Time course of the shoot length after the seed sowing. Data are means ± SE ($n = 10$). **D** Whole seedling morphology at 10 days after sowing. Bar = 1 cm

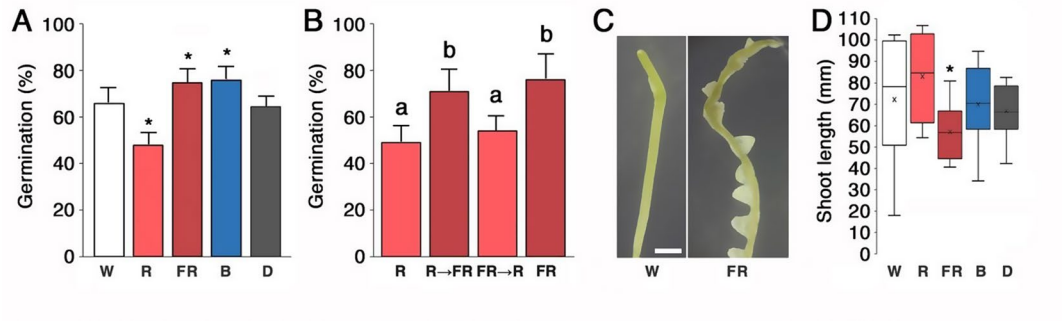

Fig. 2 Effect of different light conditions on germination and shoot growth. (**A**) Effect of light wavelengths on germination. The germination rate was measured at 3 days after the seeds were sown on MS plates and placed under continuous white (W), red (R), far-red (FR), blue (B) light or darkness (D). Data are means ± SE ($n = 3$, 20 seeds each). Asterisks indicate a significant difference from the white-light treatment ($p < 0.05$). (**B**) Effect of red and far-red light treatments on germination. The imbibed seeds were placed under red light for 48 h (R), red light for 24 h followed by far-red light for 24 h (R->FR), far-red light for 24 h followed by red light for 24 h (FR->R), or far-red light for 48 h (FR). Each treatment was followed by the dark treatment for 24 h. Data are means ± SE ($n = 3$, 20 seeds each). Different letters indicate a significant difference ($p < 0.05$) between treatments. (**C**) Shoot morphology of 10-day-old seedlings grown under white (W) and far-red (FR) light. Bar = 5 mm. (**D**) Effect of light wavelengths on shoot elongation. Three-day-old seedlings grown under 16/8 h white light/dark were transferred under continuous white (W), red (R), far-red (FR), blue (B) light or darkness (D) and grown for 7 days. Data are means ± SE ($n = 10$). An asterisk indicates a significant difference from the white-light treatment ($p < 0.05$)

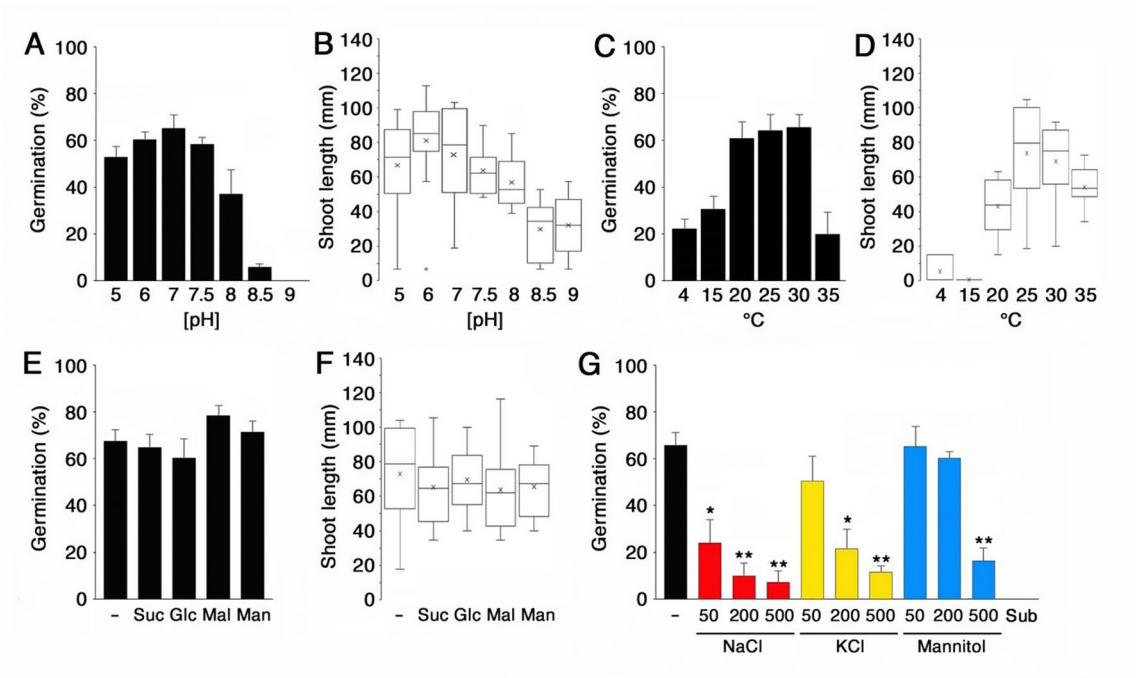

Fig. 3 Effect of pH, temperature, sugar, and high salt conditions on germination and shoot growth. **A** and **B** Effect of media pH on germination (**A**) and shoot elongation (**B**). **C** and **D** Effect of temperature on germination (**C**) and shoot elongation (**D**). MS agar plates were upside down. **E** and **F** Effect of sugars on germination (**E**) and shoot elongation (**F**). 100 mM sucrose (Suc), glucose (Glc), maltose (Mal), or mannose (Man) was added to MS agar plates. **G** Effect of high salt and osmotic stresses on germination. Data are means ± SE ($n = 3$, 20 seeds each in **A**, **C**, **E**, and **G**, $n = 10$ in **B**, **D**, and **F**). In **B**, **D**, and **F**, 3-day-old seedlings grown in control conditions were transferred to the indicated conditions and grown for 7 days. In **G**, asterisks indicate a significant difference from control (-) treatment (* $p < 0.05$, ** $p < 0.01$). Sub, submergence by water

supplementation with 100 mM sucrose, glucose, maltose, or mannose had no significant effect on either germination efficiency or shoot elongation (Fig. 3E, F). NaCl and KCl significantly inhibited germination at concentrations of 50 mM and 200 mM, respectively (Fig. 3G). On the other hand, exposure of the seeds to mannitol, used as an osmotic control, had an inhibitory effect on germination at 500 mM while complete submergence in water also fully inhibited germination (Fig. 3G).

Effect of phytohormones on germination and growth

Among the hormones examined, supplementation with 1 µM gibberellic acid (GA3) enhanced germination, while 5 µM ABA and 100 µM amino-cyclopropane carboxylic acid (ACC), a precursor of ethylene, reduced germination (Fig. 4A). Methyl jasmonate (MeJA) also enhanced germination at 1 µM, whereas 1 µM kinetin, 1 µM indole-3-acetic acid (IAA), 100 µM bikinin (an inhibitor of GSK3 kinases, which is used as a substitute for brassinosteroids, De Rybel et al. 2009), and 100 µM salicylic acid (SA) showed no significant effect on germination (Fig. 4A). When the seedlings were transferred from MS plates to plates containing different hormones after germination, shoot elongation was significantly enhanced by MeJA but repressed by IAA, ABA, ACC, and SA (Fig. 4B). GA3, kinetin, and bikinin had no significant effect on shoot elongation.

We also examined the effect of the artificial auxin, 2,4-D on seedling growth and found that 5 µM 2,4-D prolonged the survival of trichomes at the root tip, which normally decay within 10 days after germination (Fig. 4C, D). Treatment

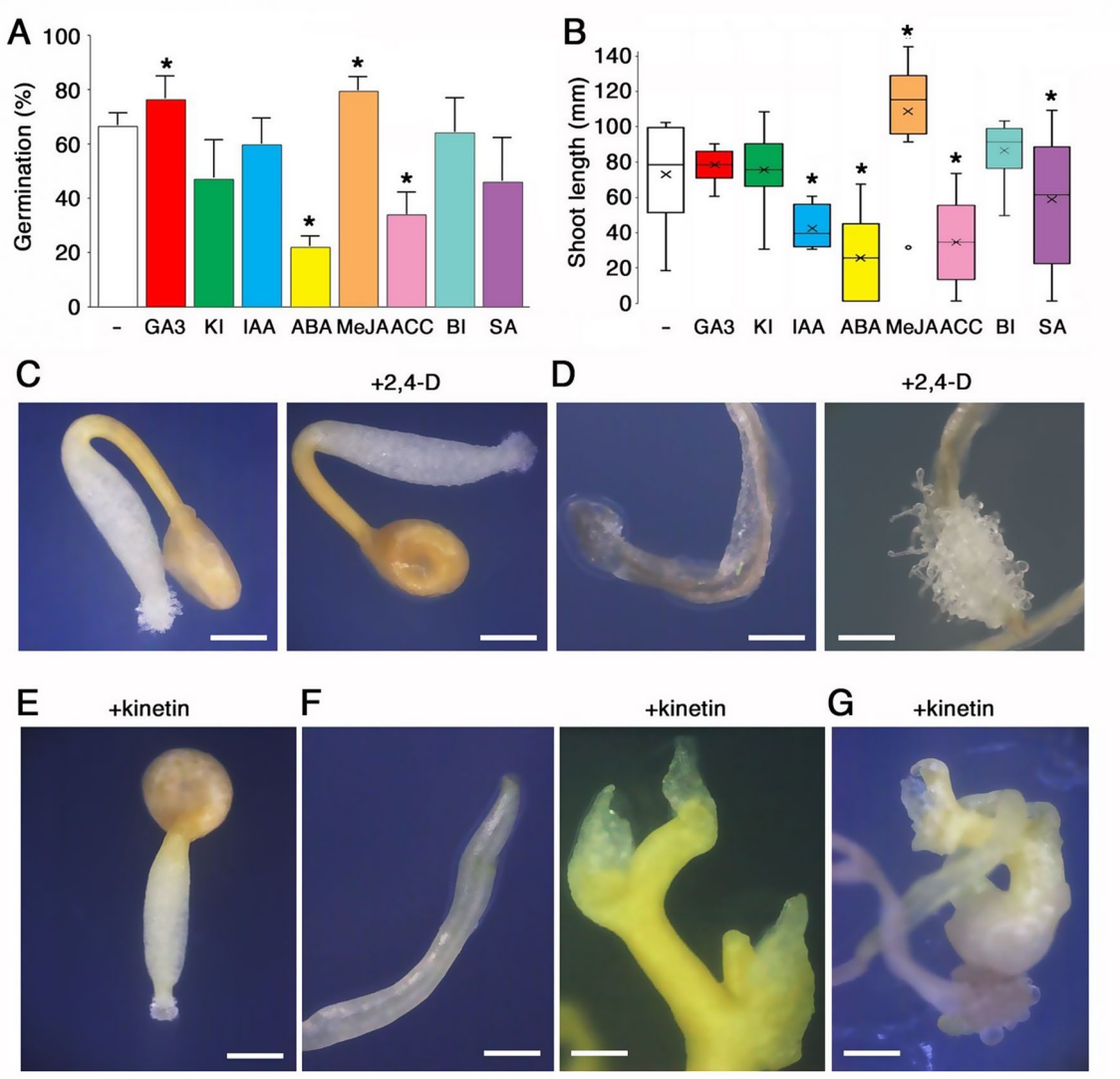

Fig. 4 Effect of phytohormones on germination and shoot growth. **A** and **B** Effect of phytohormone treatments on germination (**A**) and shoot elongation (**B**). Phytohormones and their substitutes examined were 1 µM gibberellic acid (GA3), 1 µM kinetin (KI), 1 µM indole acetic acid (IAA), 5 µM abscisic acid (ABA) 1 µM methyl jasmonate (MeJA), 100 µM amino-cyclopropane carboxylic acid (ACC), 100 µM bikinin (BI), and 100 µM salicylic acid (SA). In **B**, 3-day-old seedlings grown in control conditions were transferred to the indicated conditions and grown for 7 days. Data are means ± SE ($n = 3$, 20 seeds each in **A**, $n = 10$ in **B**). Asterisks indicate a significant difference from control (-) treatment ($p < 0.05$). **C** Morphology of 4-day-old seedlings grown without or with 5 µM 2,4-D. **D** Morphology of the root of 10-day-old seedlings grown without or with 5 µM 2,4-D. **E** Morphology of 4-day-old seedlings grown with 1 µM kinetin. **F** Morphology of the shoot of 10-day-old seedlings grown without or with 1 µM kinetin. **G** Morphology of the shoot of 20-day-old seedlings grown with 1 µM kinetin. Bars in **C** to **G** = 1 mm

with 1 μM kinetin did not induce seedling hook formation (Fig. 4C, E). Notably, kinetin treatment induced the formation of scale leaves on the shoot and the emergence of lateral shoots in 10-day-old seedlings (Fig. 4F). Kinetin treatment for 20 days resulted in the formation of callus-like cell clusters at the base of the shoot and the root tip (Fig. 4G).

Responses of seeds and seedlings to amino acids and polyamines

We next investigated the effect of exogenous amino acids on germination and shoot growth. Among the 20 amino acids tested, alanine and aspartate slightly but significantly increased the germination rate, while histidine reduced it (Fig. 5A). The germination rate at 10 days after sowing was similar between the seeds treated with no amino acids, alanine, and aspartate, but it remained low in the presence of histidine (Fig. 5B). None of the amino acids examined had a significant effect on shoot elongation (Fig. 5C).

We further found that treatment with polyamines had no apparent effect on the germination (Fig. 5D) while thermospermine inhibited shoot elongation (Fig. 5E). Thermospermine, a structural isomer of spermine, plays a role in negatively regulating xylem differentiation (Takano et al. 2012). To examine the effect of thermospermine on vascular tissues, we observed sections of the seedlings and found that vascular cell differentiation was severely repressed by thermospermine in the basal part of the shoot (Fig, 5 F). Sections of the root revealed that, although the development of vascular tissues was not affected by thermospermine, the shape of cells in the root was generally distorted in seedlings

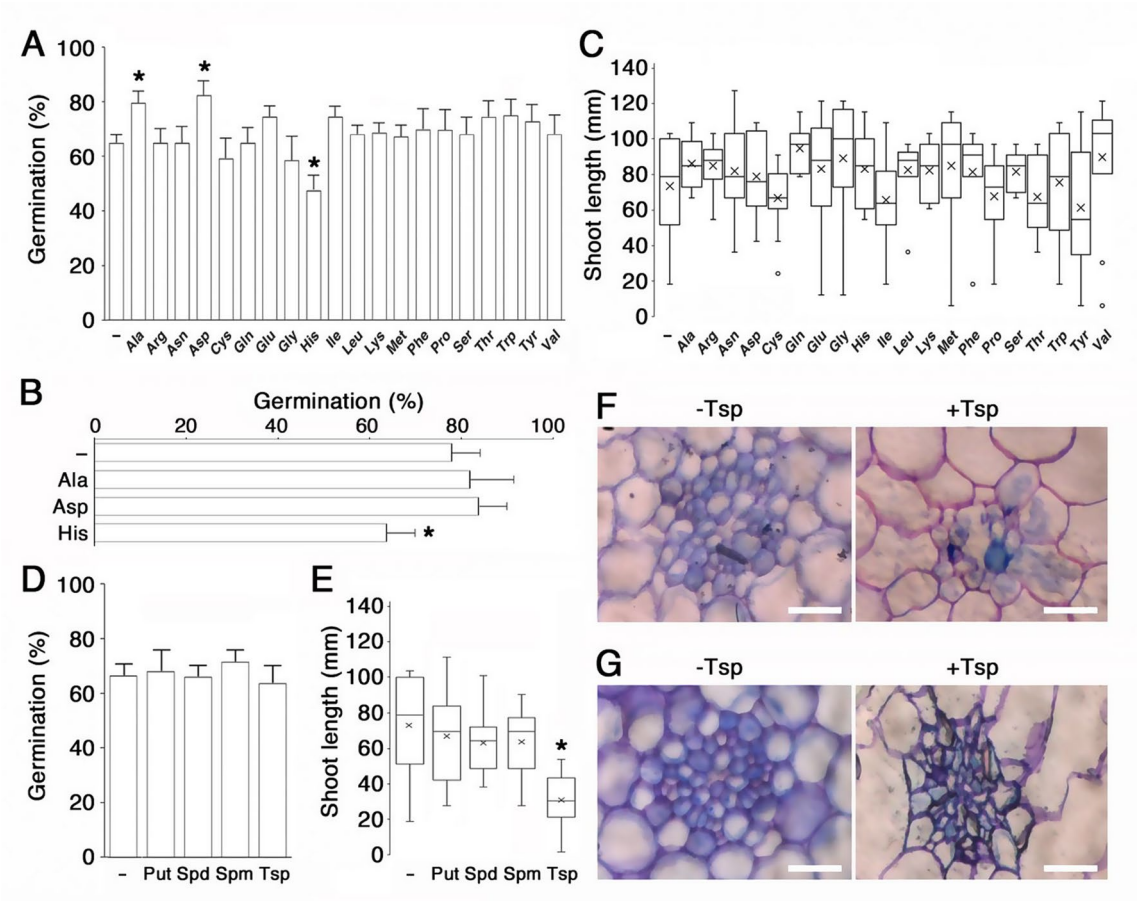

Fig. 5 Effect of amino acids and polyamines on germination and shoot growth. **A** and **B** Effect of amino acid treatments on germination. The germination rate was measured 3 days (**A**) and 10 days (**B**) after sowing the seeds on MS plates supplemented with 1 mM of the indicated amino acid. Data are presented as means ± SE ($n = 3$, with 20 seeds per replicate). Asterisks indicate a significant difference from control treatment ($p < 0.05$). **C** Effect of amino acid treatments on shoot elongation. Three-day-old seedlings grown under control conditions were transferred to the indicated treatments and grown for 7 days. Data are presented as means ± SE ($n = 10$). **D** and **E** Effect of polyamine treatments on germination (**D**) and shoot elongation (**E**). MS agar plates were supplemented with 1 mM putrescine (Put), 1 mM spermidine (Spd), 100 μM spermine (Spm), or 100 μM thermospermine (Tsp). In **E**, 3-day-old seedlings grown under control conditions were transferred to the indicated conditions and grown for 7 days. Data are presented as means ± SE ($n = 3$, with 20 seeds per replicate in **D**, $n = 10$ in **E**). Asterisks indicate a significant difference from control (-) treatment ($p < 0.05$). **F** and **G** Sections of the basal part of the shoot of 10-day-old seedlings (**F**) and the root of 5-day-old seedlings (**G**), grown without or with 100 μM thermospermine. Bars = 100 μm

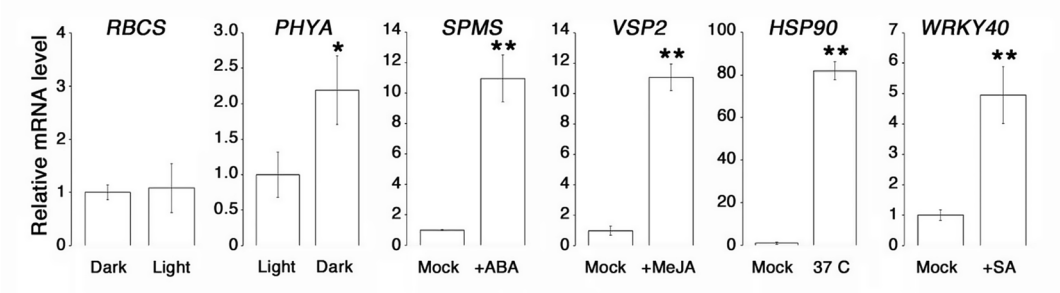

Fig. 6 Effect of light, high temperature, ABA, MeJA, and SA on the expression of the genes known to be responsive in other plants. Expressions of putative *C. campestris* homologs of *RbcS* (accession #VFQ99940), *PhyA* (accession #VFQ60648), *Spms* (accession #VFQ58264), *Vsp2* (accession #VFQ80521), *Hsp90* (accession #VFQ72691), and *Wrky40* (accession #VFQ87276) were examined by qRT-PCR. Light treatments were 24-h white light on 9-day-old dark-grown seedlings in MS agar plates and vice versa. Other treatments were 3-h 5 µM ABA, 12-h 1 µM MeJA, 3-h heat shock at 37 °C, and 12-h 100 µM SA on seedlings grown in MS plates for 9 days and pre-incubated for 24 h in MS liquid media. *EF1α* (accession #VFQ91475) was used as the reference gene. Transcript levels relative to control treatment are shown. Data are means ± SE of three different experiments. Asterisks indicate a significant difference from control treatment (* $p < 0.05$, ** $p < 0.01$)

treated with thermospermine (Fig, 5G), suggesting an accelerated degeneration of the root tissue.

Environmental responses of gene expression in the seedling

We finally addressed whether the molecular responses to external stresses have been evolutionarily retained in *C. campestris* seedlings. Quantitative RT-PCR experiments revealed that the expression of an *RbcS* homolog, which putatively encodes a small subunit of Rubisco in the chloroplast, was not upregulated after transfer from dark to white light. A homolog of *Phytochrome A* (*PhyA*), whose expression is generally upregulated in the dark, showed an increased expression after 24 h of dark treatment (Fig. 6). We confirmed that the expression of homologs of ABA-responsive *SPMS*, encoding spermine synthase (Yariuchi et al. 2021), salicylic acid (SA)-responsive *VSP2* (Koornneef et al. 2008), MeJA-responsive *WRKY18* (Potschin et al. 2014), and the heat-shock protein gene, *HSP90*, were all increased by treatment with ABA for 24 h, SA for 24 h, MeJA for 24 h, and heat stress at 37 °C for 4 h (Fig. 6).

Discussion

Light quality and duration are pivotal in triggering the twining response and haustorium formation in dodders (Tada et al. 1996; Orr et al. 1996; Haidar 2003). A study on *Cuscuta japonica* Choisy. has revealed that blue light is essential for twining and a lower R/FR ratio is important for subsequent haustorium induction (Furuhashi et al. 2021). *Cuscuta epilinum* Weihe. seedlings can respond to remarkably small-scale variations in R/FR signals and thereby potentially discriminating between hosts that differ in proximity or architecture (Smith et al. 2021). Blue light-dependent twining may be a form of blue light-mediated phototropic responses. Both far-red light and touch stimuli play a critical role for the haustorium formation as a signal showing the presence of putative host plants. Low R/FR irradiation has been shown to induce the expression of genes related to cell wall degradation in *Cuscuta chinensis* Lam. (Pan et al. 2022). Stage-specific genes during far-red light-induced haustorium formation have been identified in *C. campestris* (Bawin et al. 2022). Another study in *C. campestris* has shown that mature shoots exhibit coiling around the host and haustorium formation even under red light, suggesting that the response to red light is modified during the transition from young to mature shoots to facilitate parasitism (Yokoyama et al. 2023). In the present study, we found that seed germination is also stimulated by far-red light and reduced by red light. We further confirmed that, although not completely, the germination response to red and far-red light is reversible, suggesting the involvement of phytochrome signaling in germination. This response is apparently opposite to that observed in many plant species, where germination is induced or promoted by a single red-light pulse. Germination in some plant species requires only rehydration in the dark during which phytochromes may be converted into the active far red-absorbing form (Pfr). In tomato, germination is inhibited not only by far-red light but also by strong continuous red light (Shichijo et al. 2001). This inhibition is suggested to be caused by degradation of the active but light unstable Pfr of PhyA (Appenroth et al. 2006). Given the reversed response to red and far-red light, *C. campestris* seeds may possess a unique phytochrome signaling mechanism for germination, differing from the conventional pathway followed by both light- and dark-germinators. Far red-light induced germination was first reported for a bromegrass, *Bromus sterilis* (Hilton 1982). Interestingly,

the similar response is observed in another holoparasitic plant, *Orobanche coerulescens* (Takagi et al. 2009). Thus, the mechanism may have been evolved independently in some families, although the causal relationship between the evolution of parasitism or the loss of photosynthesis and the reversal of the phytochrome action remains unknown. Shoot elongation is also reduced by far-red light in *O. coerulescens* (Takagi et al. 2009), suggesting a common alteration of phytochrome signaling components in these parasitic plants. Alternatively, the reduction in the shoot length under far-red light observed in *C. campestris* might be explained by a trade-off with haustorium formation.

Among abiotic stresses examined in this study, pH values higher than 7 was found to have a severely inhibiting impact on germination. A similar response to alkaline conditions and the preference to acidic conditions in seed germination have been reported for some species of the *Ipomoea* genus (Oliveira and Norsworthy 2006; Singh et al. 2012). Hence, alkaline soil pH is likely to be a limiting factor for germination, a condition generally common to the morning glory family, Convolvulaceae. Our results show that the germination rate was highest at 30 °C and was markedly reduced below 20 °C are consistent with previous reports on the effect of temperature on field dodder germination (Hutchison and Ashton 1980; Benvenuti et al. 2005; Goldwasser et al. 2016). A similar trend was observed in shoot elongation. These traits seem to reflect the tropical origin of dodders.

Notably, in hormone experiments, MeJA promoted both seed germination and shoot elongation. Many studies have shown that jasmonates induce defense responses against pathogens but have negative effects on plant growth and germination (Linkies and Leubner-Metzger 2012; Ghorbel et al. 2021; Pan et al. 2023). Jasmonate levels are closely correlated with the accumulation of ABA in *Arabidopsis* seeds (Dave et al. 2016). JASMONATE RESISTANT 1 (JAR1), which encodes an enzyme that produces a bioactive molecule, jasmonoyl-isoleucine in *Arabidopsis*, and is also known as FAR-RED INSENSITIVE 219 (FIN219), participates in PhyA-mediated far-red light signaling (Chen et al. 2015). It is thus possible that JAR1/FIN219 plays a role in germination activated by far-red light and MeJA in *Cuscuta*. However, there are some plants that exhibit jasmonate-stimulated germination, such as pear (Yildiz et al. 2008), apple (Górnik et al. 2018), and wheat (Xu et al. 2016). Further research is needed to evaluate the mechanism of jasmonate signaling and its crosstalk with light and other hormone pathways in *Cuscuta*.

Our results also revealed that *C. campestris* can develop scale leaves without haustorium formation when treated with kinetin. The degree of scale leaf development may vary among *Cuscuta* species. The shoot tips of *C. japonica*, *C. australis*, and *C. chinensis* have several spirally arranged minute scales (Fujita 1964). Although a study indicated that the seeds of *C. campestris* contain approximately 1 μM zeatin (Al-Gburi et al. 2019), it remains to be examined whether the degree of scale leaf development is attributed to the endogenous level of cytokinin. Taking previous research into account (van der Kooij et al. 2000; Vogel et al. 2018), retrogression of leaf development may be tightly associated with the level of host dependence or the degree of photosynthetic deficiency.

We further found that thermospermine drastically repressed the development of xylem vessels in elongating shoots, confirming its role in negatively regulating xylem formation (Takano et al. 2012). However, we observed no such effect in the root section; instead, we observed distortion of cell shapes. This is probably because the root formed during embryogenesis undergoes little or no cell division or differentiation after germination in this plant. Distorted cell shapes are a symptom of tissue degeneration. Reduced xylem development in the shoot could lead to reduced absorption of water and nutrients, which could consequently accelerate root tissue degeneration and inhibit shoot elongation.

On the other hand, among the amino acids examined, alanine and aspartate accelerated germination. Alanine is converted with 2-oxoglutarate to pyruvate and glutamate by alanine aminotransferase (AlaAT/ALT). The long seed dormancy in wild-type barley has been shown to be linked to the recessive allele of *AlaAT* (Sato et al. 2016). A positive effect of alanine on germination detected in *Cuscuta* might be due to the function of AlaAT in breaking dormancy. Aspartate is converted with 2-oxoglutarate to oxaloacetate and glutamate, or is synthesized conversely by Aspartate aminotransferase (GOT1/AAT). This enzyme plays a key role in regulating carbon and nitrogen metabolism in many organisms. During *Arabidopsis* germination, aspartate increases the most among all amino acids (Fait et al. 2006). Given that both AlaAT and GOT1 are directly involved in providing substrates for the TCA cycle (Han and Yang 2015), it is possible that supplementation with alanine and aspartate is effective in generating sufficient energy via the respiratory chain for germination. Or otherwise, pyruvate produced by AlaAT and aspartate could serve as a substrate for gluconeogenesis (Walker et al. 2021) and sugar supply via the glyoxylate cycle (Graham 2008), respectively. In contrast, only histidine was shown to be inhibitory to germination. Negative effect of histidine on germination has also been reported for *Orobanche ramose* (Vurro et al. 2006). Histidine promotes plant seed oil deposition through ABA biosynthesis and β-oxidation (Ma and Wang 2016). It remains to be determined whether exogenous histidine activates genes of ABA biosynthesis in imbibed seeds of *Cuscuta* or not. Considering a role of histidine in metal ion chelation

(Seregin and Kozhevnikova 2021), metal ion homeostasis might be seriously affected by excess histidine.

In conclusion, the results of our study revealed unique responses to environmental conditions in *C. campestris* seeds and seedlings prior to parasitism. In particular, the enhancing effect of far-red light and MeJA on seed germination may be closely related to the parasitic strategy of this plant. The molecular mechanisms underlying these responses require further investigation. In this study, we used Arabidopsis as a host plant in the growth chamber, which has small individuals and a short lifespan, and focused only on environmental factors commonly addressed in physiological plant research. It is possible that environmental factors could affect seed germination depending on the host species. Further exploration of other biologically derived factors and environmental conditions, as well as screening for bioactive molecules through chemical biology, may provide more insights into the seed germination characteristics and seedling growth traits of dodders.

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Author contributions All authors planned the study and KN performed all the experiments. KN and TT wrote the main manuscript text and KN prepared all figures. All authors reviewed the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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