

## DNA uptake by *Arabidopsis* induces changes in the expression of *CLE* peptides which control root morphology

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The presence of externally supplied DNA in the growth medium enhances growth of lateral roots and root hairs in *Arabidopsis*. This phenomenon cannot be attributed to phosphorus (P) limitation because it is independent of the plants' P status. Rather, we hypothesized that DNA triggers a currently unknown signaling pathway. Analyzing the transcriptional changes of genes induced by externally supplied DNA, we show that 7 of the 17 studied *CLAVATA3/ESR*-related (*CLEs*) genes were differentially regulated. *CLEs* are known peptide hormones that affect plant development including root morphology. While previous research had shown that overexpression of these *CLE* genes alters root morphology, changes in gene expression had not been linked to environmental triggers. The differential expression of these *CLEs* genes and accompanied changes of the root phenotype are indicative of a DNA-elicited signal pathway which affects root development. We conclude that DNA acts as a signaling compound which induces root proliferation in a way that would enhance the plant's ability to acquire nutrients from soil organic matter. Our study further confirms the importance of *CLEs* for controlling root morphology in response to specific environmental conditions, and draws attention to a novel role of DNA as a signaling compound.

to incorporate large organic molecules including proteins and DNA into roots.<sup>1,2</sup> This contradicts the notion that large organic molecules become available for plant use only after microbial conversion into small molecules and ions.<sup>3-5</sup> In *Arabidopsis*, supply of protein (bovine serum albumin) in the growth medium could only partially compensate for the lack of inorganic nitrogen in the growth medium.<sup>1</sup> In contrast, DNA fully substituted for the absence of inorganic P ( $P_i$ ) in the growth medium,<sup>2,6</sup> implying that *Arabidopsis* is well adapted to use DNA as an alternative source of P for growth.

Surprisingly, the presence of DNA in  $P_i$ -containing growth medium also enhanced growth of lateral roots and root hairs although plants were P replete,<sup>2</sup> suggesting that DNA or its degradation products act as signaling molecules that trigger changes in root morphology.<sup>2</sup> To investigate the hypothesis that DNA is a signaling compound that affects root morphology, we analyzed gene expression of plants grown with and without DNA in the growth medium. *Arabidopsis* plants, grown in axenic hydroponic culture for 3 weeks with  $P_i$  supply (5.7  $\mu\text{g KH}_2\text{PO}_4$  per mL medium) were incubated for a further 24 h without (-DNA) or with (+DNA, 0.8 mg herring sperm DNA per mL). RNA was extracted from roots to probe an Agilent microarray (Agilent Technologies, USA). Comparative analysis of gene expression between treatments revealed that DNA addition induced changes in expression of *CLAVATA3/ESR*-related (*CLE*) genes which have a known role in root morphogenesis (Fig. 1A). Microarray data were validated by quantitative real-time PCR

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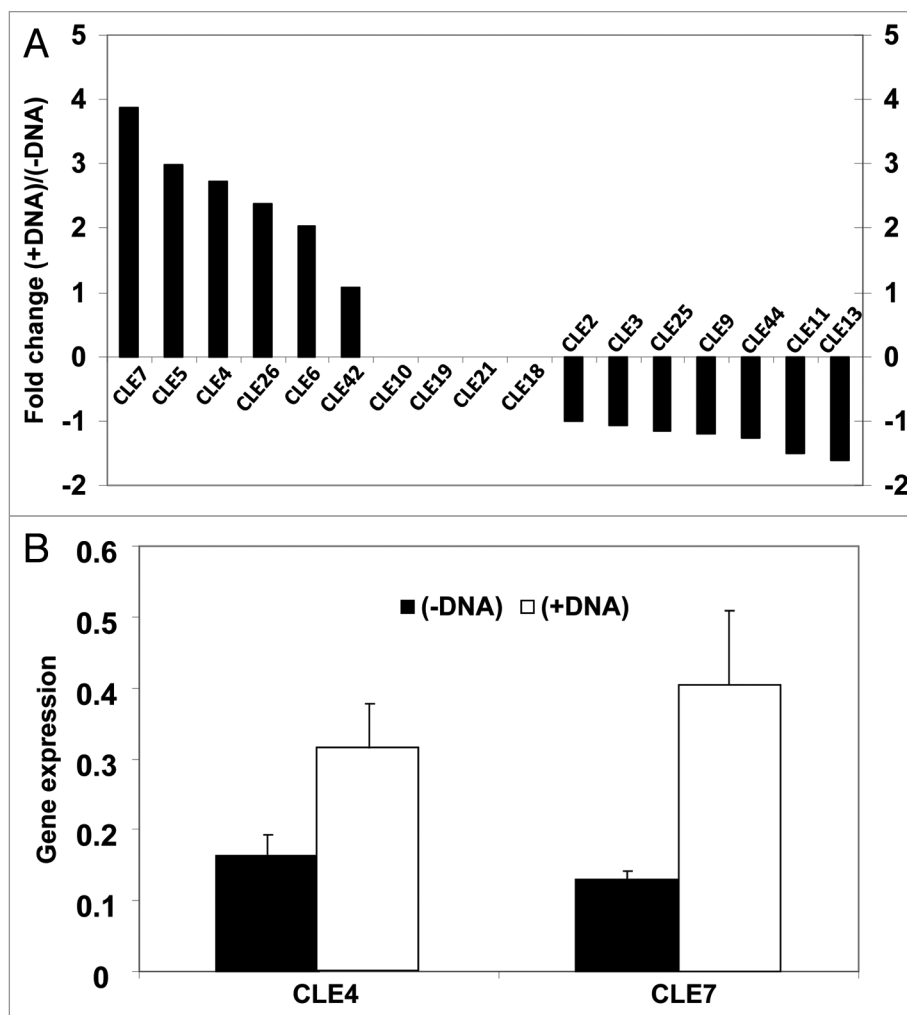
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New evidence of how plants acquire nutrients has emerged indicating that plants do not only rely on small molecules or ions to supply mineral nutrients for growth. We recently showed that plants are able



**Figure 1.** Regulation of *CLE* genes expression in Arabidopsis by DNA in the growth medium. (A) Microarray results showing that *CLE* genes are differentially regulated in the presence of DNA. Microarray data are means of two technical replicates. (B) Quantitative real-time PCR (QRT-PCR) analysis of *CLE4* and *CLE7* expression in the presence of DNA of Arabidopsis grown in the same condition as for the microarray. QRT-PCR showed 2-fold and 3.1-fold upregulation of *CLE4* and *CLE7* respectively, confirming the microarray results which show 2.7-fold and 3.9-fold upregulation, respectively. QRT-PCR results are means ( $\pm$ SD) of three independent biological assays and each value is the mean of three technical preparations. Expression levels were normalized using  $\beta$ -actin.

analysis of *CLE4* and *CLE7* expression as representative *CLE* genes with strongly increased expression (Fig. 1B).

*CLE* genes encode a family of at least 32 peptide ligands involved in plant developmental processes including regulation of root and shoot apical meristems.<sup>7-9</sup> Overexpression of certain *CLE* genes increases or shortens the primary root length of Arabidopsis seedling.<sup>10</sup> The observed changes in the expression of *CLE* genes (Fig. 1A) may therefore be responsible for, or contribute to, the changes in root morphology observed when DNA is present in the growth medium.<sup>2</sup>

The five *CLE* genes which were significantly upregulated in the +DNA

treatment (*CLE7*, AT2G31082; *CLE5*, AT2G31083; *CLE4*, AT2G31081; *CLE26*, AT1G69970; and *CLE6*, AT2G31085; Fig. 1A) induced longer primary roots in Arabidopsis seedling when overexpressed.<sup>10</sup> In contrast, *CLE13* (AT1G73965) and *CLE11* (AT1G49005) that were significantly downregulated in the +DNA treatment (Fig. 1A) caused shorter primary roots in Arabidopsis seedlings when overexpressed.<sup>10</sup> Strabala et al. (2006) reported only the effect of overexpression of *CLE* genes on primary root length of seedlings, while we observed a major stimulatory effect of externally supplied DNA on lateral root number, lateral root length, and root hair length in

mature Arabidopsis plants.<sup>2</sup> Nonetheless, our microarray expression data and the findings of Strabala et al. (2006) are consistent with the notion that changes in *CLE* genes expression mediate all or some of the effects caused by externally supplied DNA on root morphology.

Our results add important knowledge to the discussion of the role of *CLE* genes for plant development. A recurring issue in the study of *CLE* genes is the lack of phenotypes associated with mutations of individual *CLE* genes.<sup>9</sup> Apart from the possible redundancy among the *CLE* genes, a proposed explanation is that knock-out-phenotypes are only visible under certain environmental conditions.<sup>9</sup>

Our findings strongly support this possibility as they demonstrate that a set of *CLEs* is differentially regulated in response to the environmental stimulus exerted by DNA and results in a co-ordinated alteration of root morphology. Therefore, plants grown in the presence of DNA may offer a model system to study the role of individual *CLEs* genes.

In conclusion, our results indicate that in *Arabidopsis* a series of *CLE* genes control root morphogenesis under specific environmental conditions. It is tempting to argue that the high number of possible combinations of the 32 *CLEs* allow plants to respond to specific growth conditions, such as different nutrient supply situations in soil. Our results are also relevant for the understanding of how plants control root growth in soils that contain organic

forms of nutrients. The complex interface between plants, microbes and soil is not well understood and plants may have evolved specific pathways that allow controlling organ-morphogenesis in response to the presence of different forms of mineral nutrients.

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