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REVIEW PAPER

The underground life of homeodomain-leucine zipper transcription factors

María Florencia Perotti, Agustín Lucas Arce, and Raquel Lía Chan*

Instituto de Agrobiotecnología del Litoral, CONICET, Universidad Nacional del Litoral, FBCB, Colectora Ruta Nacional 168 km 0, 3000 Santa Fe, Argentina

*Correspondence: rchan@fbcb.unl.edu.ar

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Abstract

Roots are the anchorage organs of plants, responsible for water and nutrient uptake, exhibiting high plasticity. Root architecture is driven by the interactions of biomolecules, including transcription factors and hormones that are crucial players regulating root plasticity. Multiple transcription factor families are involved in root development; some, such as ARFs and LBDs, have been well characterized, whereas others remain less well investigated. In this review, we synthesize the current knowledge about the involvement of the large family of homeodomain-leucine zipper (HD-Zip) transcription factors in root development. This family is divided into four subfamilies (I–IV), mainly according to structural features, such as additional motifs aside from HD-Zip, as well as their size, gene structure, and expression patterns. We explored and analyzed public databases and the scientific literature regarding HD-Zip transcription factors in Arabidopsis and other species. Most members of the four HD-Zip subfamilies are expressed in specific cell types and several individuals from each group have assigned functions in root development. Notably, a high proportion of the studied proteins are part of intricate regulation pathways involved in primary and lateral root growth and development.

Keywords: HD-Zip, homeodomain-leucine zipper, root atlas, root branching, root development, transcription factor.

Introduction

The roots are the organs of anchorage, water, and nutrient uptake

Plants, being sessile organisms, have a plastic development that allows them to change their morphology and adapt to their environment. As anchorage organs, the roots alter their development, extending through the soil to optimize water and nutrient uptake (de Dorlodot *et al.*, 2007). However, genetic factors are also important determinants of root growth, as clearly evidenced by the different main root-system morphologies present in monocots, which have many adventitious postembryonic roots derived from the shoot in parallel to the primary root, and in dicots, in which there is a dominant primary root from which lateral roots develop (Osmont *et al.*, 2007; Atkinson *et al.*, 2014).

After seed germination, the stem-cell niche cells start to divide and differentiate, resulting in the root meristem, which

Abbreviations: HD-Zip, homeodomain-leucine zipper; TF, transcription factor; GUS, β-glucuronidase.

contains three different zones: the meristematic, elongation, and differentiation zones. In addition, a transition zone lies between the meristematic and elongation zones, which is modulated by the phytohormones auxin and cytokinin (Salvi *et al.*, 2020). The root apical meristem, which is established during embryogenesis, contains stem cells surrounding the quiescent center (QC), a group of cells forming a hemisphere with the flat face toward the root tip, where cell division proceeds very slowly or not at all after embryogenesis (Clowes *et al.*, 1958; Taiz and Zeiger, 2006). Stem cells on the rootward side of the QC produce the columella root cap, while lateral and shootward stem cells generate the vascular, endodermal, cortical, epidermal, and lateral root cap cells (reviewed by Petricka *et al.*, 2012).

The pathways underlying root plasticity arise from the interaction of many biomolecules, among which transcription factors (TFs) and hormones are key actors. In the complex molecular events that determine root architecture and regulate plasticity in response to environmental factors, TFs from different families have specific functions, playing critical roles in development and/or adaptation (Gonzalez, 2016; Hong, 2016; Li *et al.*, 2020*b*; Renau-Morata *et al.*, 2020).

Among the TFs, members of the ARF and LBD families play a central role in lateral root development as well as in phytohormone crosstalk (Friml *et al.*, 2003; Lee *et al.*, 2009; Lavenus *et al.*, 2013; Banda et al., 2019; Xu *et al.*, 2020). These TFs are clearly critically important but they do not act alone in root development and adaptation.

The discovery of the HD-Zip family, unique to plants

Homeodomains (HDs) were first discovered in animals (Gehring, 1987) and a few years later, the first plant TF possessing an HD was discovered in maize. The ectopic expression of this TF, encoded by the *Knotted1* gene, produced knotting in leaves (Vollbrecht *et al.*, 1991). Almost simultaneously, the existence of a new class of TFs unique to plants, the homeodomain-leucine zipper (HD-Zip) proteins, was discovered (Ruberti *et al.*, 1991). In these TFs, the HD is the DNA-binding domain, whereas leucine zippers (Zip or LZ) are dimerization motifs characterized by the presence of a leucine (or isoleucine) residue every seven amino acids.

Compared with organisms from other kingdoms, plants exhibit great plasticity to adapt to environmental conditions; this ability is governed, at least in part, by transcriptional programs in which TFs are key players. Therefore, as HD-Zip TFs are exclusive to the plant kingdom, they were postulated to be associated with environmental responses (Ruberti *et al.*, 1991; Schena and Davis, 1992).

Since their discovery, knowledge about the HD-Zip TFs has increased substantially, and these proteins have been connected with numerous developmental events beyond those related to environmental and stress conditions such as drought, salinity, flooding, and UV radiation (Mukherjee *et al.*, 2009; Harris *et al.*, 2011; Turchi *et al.*, 2015; Perotti *et al.*, 2017; Sessa *et al.*, 2018; Kovalchuk *et al.*, 2019; He *et al.*, 2020). Despite the availability of these reports about the involvement of HD-Zip members in plant stress responses, many members of the family are still poorly characterized, if at all, and others have been only partially studied. In particular, knowledge about their non-stress-related functions in plant development, and especially their role in root growth and branching, is limited. This limitation is particularly prominent for species not considered model plants.

HD-Zip subfamilies I–IV: structure and assigned functions in the plant kingdom

Many proteins with an HD-Zip domain have been identified and they have been classified into four subfamilies, I–IV (Schena and Davis, 1992; revised by Capella *et al.*, 2016). The proteins in the different subfamilies differ significantly in their structure, patterns of expression, environmental responses, and functions. Within some subfamilies, there are further classifications (described below) according to the presence of additional motifs, which likely led to neofunctionalization (Henriksson *et al.*, 2005; Arce *et al.*, 2011). Moreover, as with other gene families, the HD-Zip TFs exhibit several pairs of paralogous genes that have arisen from a common ancestral gene by duplication events. According to whether these events are recent or older, there is a higher or lower likelihood that the paralogs retain common functions.

In this review, we synthesize current knowledge about TFs belonging to the HD-Zip family that participate in root development.

HD-Zip TFs in Arabidopsis

HD-Zip TFs are modular proteins that possess a combination of domains and motifs, but all have in common the adjacent HD and a LZ domain that define them. Among other domains and motifs found in HD-Zip TFs are the CPSCE motif (named based on the one-letter codes for the five conserved amino acids cysteine, proline, serine, cysteine, and glutamic acid; Tron *et al.*, 2002), the AHA motif (aromatic and large hydrophobic residues embedded in an acidic context), the ZIBEL motif (which contains an LxLxL motif), the START domain (steroidogenic acute regulatory protein-related lipid transfer), the SAD domain (START-adjacent domain), and the MEKHLA domain (named based on the one-letter codes for the highly conserved amino acids methionine, glutamic acid, lysine, histidine, leucine, and alanine). These motifs are located upstream or downstream of the HD-Zip domain and some are subfamily-specific (Mukherjee and Burglin, 2006; Ariel *et al.*, 2007).

Members of subfamily I are relatively small proteins (~30–35 kDa) with the HD-Zip roughly in the middle of the molecule, flanked by an amino- and a carboxy-terminus containing

several conserved motifs. In Arabidopsis, this subfamily has 17 members, as shown in Fig. 1. Based on a phylogenetic analysis, they were classified into α, β, γ, δ, ε, and θ groups (Henriksson *et al.*, 2005), which was supported by their exon–intron structure. A later multispecies phylogenetic analysis, which also considered conserved motifs (e.g. the AHA motif and those without known function) present in the carboxy-terminus and shared between members of a group, divided the proteins into six clades, I–VI (Arce *et al.*, 2011; revised by Capella *et al.*, 2016).

Subfamily II includes 10 members (Ciarbelli *et al.*, 2008) that, in addition to the HD-Zip domain, have two more motifs: the CPSCE motif, which is adjacent to and downstream of the LZ, and an N-terminus consensus sequence, the ZIBEL motif (Fig. 1). Subfamily III is the smallest, with only five members in Arabidopsis (Fig. 1). The binding domain of these proteins has four extra amino acids between the HD and the LZ. In addition, they contain the START, SAD, and MEKHLA domains (Mukherjee *et al.*, 2006). Subfamily IV comprises 16 members in Arabidopsis; the most distinguishable features of these proteins are the presence of a loop in the middle of the LZ domain and the lack of a MEKHLA domain compared with the subfamily III members (Nakamura *et al.*, 2006; Fig. 1). Details of the structural features of the four subfamilies, as well as phylogenetic trees, have already been reviewed by several authors (Floyd *et al.*, 2006; Ariel *et al.*, 2007; Ciarbelli *et al.*, 2008; Mukherjee *et al.*, 2009; Bürglin, 2011; Capella *et al.*, 2016).

Hidden treasures in public databases: HD-Zip members expressed in Arabidopsis roots

Functional studies about HD-Zip TFs in roots are scarce, and only a few genes of this family have been functionally characterized in Arabidopsis. However, public databases have greatly increased and have documented knowledge about the expression patterns of plant genes, which allows us to infer that many members of this TF family, including the four subfamilies, could be involved in root development in Arabidopsis. At least three key sources are available: BAR from the University of Toronto (http://bar.utoronto.ca/; Brady *et al.*, 2007; Kilian *et al.*, 2007; Dinneny *et al.*, 2008); the Root Atlas (Zhang *et al.*, 2019; http://wanglab.sippe.ac.cn/rootatlas); and the Plant sc-Atlas, which was very recently released by Ghent University (Wendrich *et al.*, 2020; https://bioit3.irc.ugent.be/plant-sc-atlas/).

These databases have compiled information obtained from many microarrays and RNA-Seq analyses performed by different research groups. Using the BAR and Root Atlas databases, we investigated the expression patterns of genes encoding HD-Zip proteins from the four subfamilies. To do these analyses, we retrieved all the primary data related to genes encoding HD-Zip TFs and analyzed this information, using the method described in Appendix S1 at Dryad Digital Repository, https://doi.org/10.5061/dryad.mpg4f4qzb. Additionally, we retrieved the expression levels for marker TFs with known functions in specific root tissues or cell types. Based on this information, we set expression thresholds that allowed us to annotate tissues in which HD-Zip TF expression levels are equal or greater to those of functional TFs. We used this evidence as an indication that an HD-Zip TF is potentially functional in a tissue or cell type.

Expression of HD-Zip I members

The first comprehensive study of the expression of HD-Zip I members in roots was carried out by Henriksson *et al.* (2005), using non-quantitative reverse transcription (RT)–PCR. The authors isolated RNA from 12-day-old Arabidopsis roots and detected positive signals for *AtHB1*, *AtHB3*, *AtHB20*, *AtHB23*, *AtHB5*, *AtHB6*, *AtHB16*, *AtHB7*, *AtHB12*, *AtHB21*, *AtHB40*, *AtHB53*, *AtHB51*, and *AtHB52*; transcripts corresponding to *AtHB13*, *AtHB22*, and *AtHB54* were not detected in the 12-day-old roots. However, *AtHB13* and *AtHB22* were expressed in younger seedlings (5 days old), although *AtHB54* was not (Henriksson *et al.*, 2005). Even though the technique used was not quantitative, this study provided the first indication about the role of HD-Zip I members in root development.

Currently, the BAR database indicates that all HD-Zip I members other than *AtHB22* and *AtHB54* are expressed in roots of seedlings grown in normal conditions (Figs 2, 3; Tables S1, S2 at Dryad). Indeed, BAR does not have any information about the expression of *AtHB22* and *AtHB54*. When plants were subjected to different treatments, such as salt, drought, UV-B, or other stress conditions, the expression patterns of most of these genes changed (Fig. 4).

Our in-depth search of the databases showed that, in normal conditions, many members were expressed in the lateral root cap, and fewer in the columella and the QC. The subfamily was also represented in the cortex and endodermis (Fig. 2; Table S2 at Dryad).

The only subfamily I members detected in the xylem were *AtHB1*, *AtHB5*, *AtHB6*, *AtHB12*, and *AtHB16*, and in the procambium *AtHB1*, *AtHB16*, *AtHB51*, and *AtHB53*. Twelve members were found to be expressed in the xylem-pole pericycle, and nine in the phloem-pole pericycle (Fig. 3A; Table S2 at Dryad). Considering cell types specialized in root hair and lateral root development, several members were detected in both the root hair and non-hair epidermal cells; among them, *AtHB20* exhibits the highest levels (Fig. 3B; Table S2 at Dryad). The genes expressed in the lateral root primordium are different from those detected in root hair (Fig. 3C; Table S2 at Dryad).

When plants were subjected to abiotic stress factors, the scenario became more complex; each member appeared to be up-regulated or down-regulated by various stress treatments. *AtHB1* was induced by osmotic, genotoxic, UV-B, and high-temperature conditions, whereas it was slightly repressed by sodium chloride. The paralogous genes *AtHB13* and *AtHB23* were repressed by osmotic and salinity stresses, whereas *AtHB5*

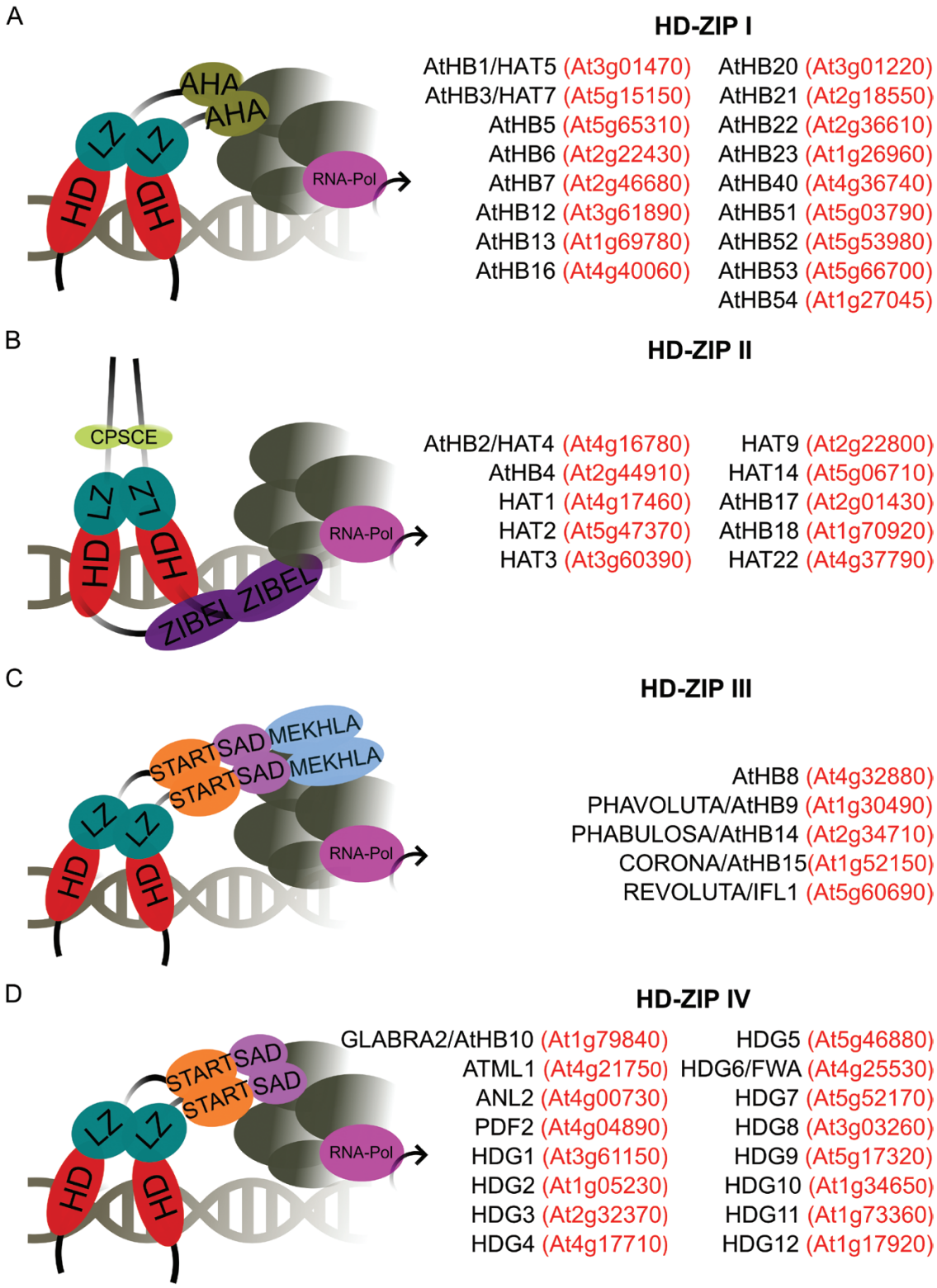

Fig. 1. The HD-Zip TF subfamilies. (A) HD-Zip I; (B) HD-Zip II; (C) HD-Zip II; (D) HD-Zip IV. The left panels show a schematic structure of proteins from each subfamily, including motifs additional to the HD-Zip that characterize them. The right panels show the name and (in red) ID of each member.

transcripts were induced by the same factors. The paralogs *AtHB6* and *AtHB16*, as well as *AtHB21*, were regulated by almost all the treatments, albeit to different extents. *AtHB7* and its paralog *AtHB12*, which were weakly expressed in roots, were up-regulated by cold temperatures and by osmotic and salinity stresses. The paralogous genes *AtHB40* and *AtHB53*

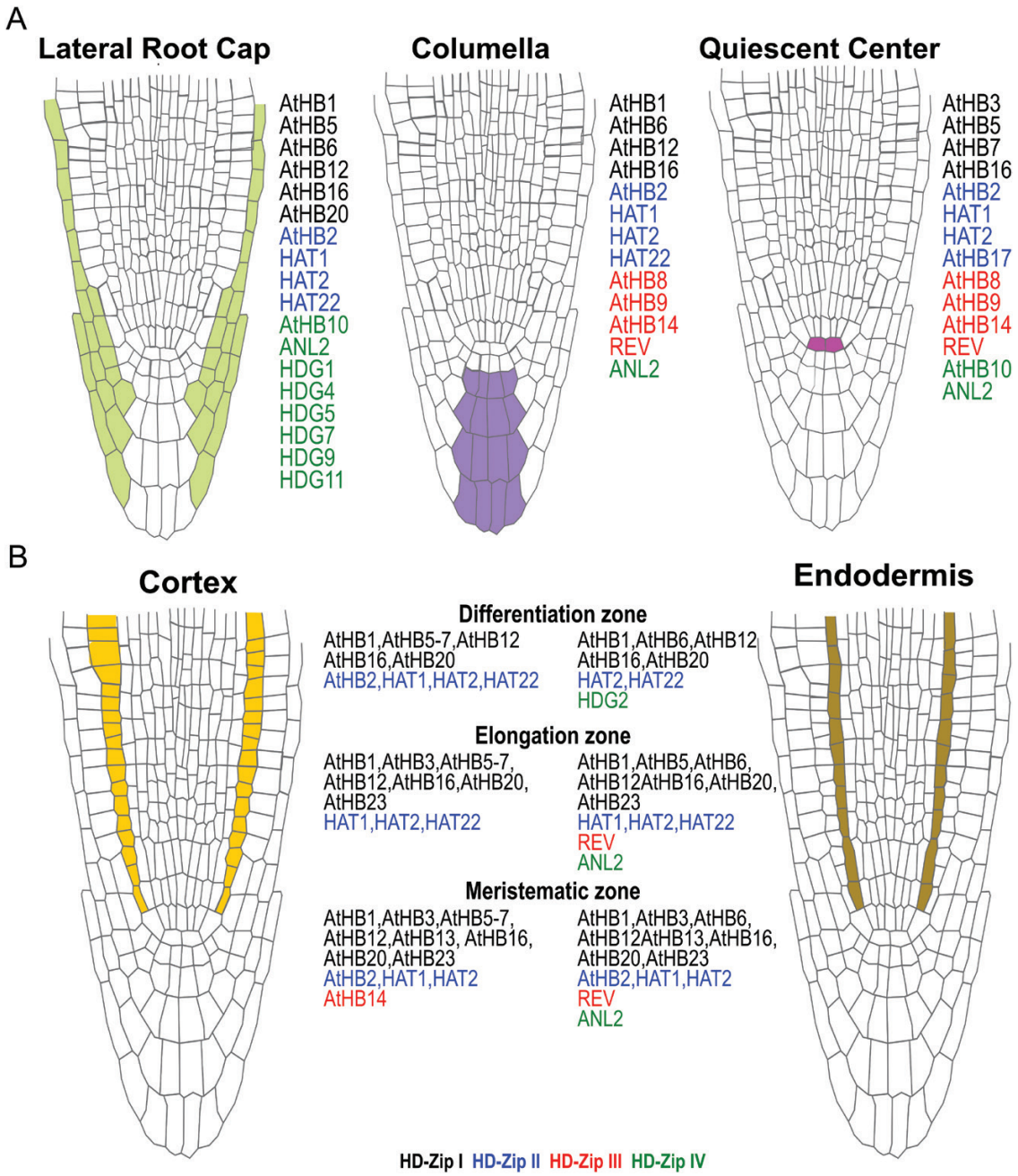

Fig. 2. Genes encoding HD-Zip TFs expressed in the root tip. (A) Schematic representation of different root tissues of the root tip: lateral root cap, columella, and quiescent center. (B) Drawings of longitudinal root sections of cortex and endodermis tissues, showing the meristematic, elongation, and differentiation zones. The HD-Zip members expressed in each tissue are listed and color coded: HD-Zip I (black), HD-Zip II (blue), HD-Zip III (red), HD-Zip IV (green). Key source: BAR from the University of Toronto (http://bar.utoronto.ca/; Brady *et al.*, 2007; Kilian *et al.*, 2007; Dinneny *et al.*, 2008). Complete data and analysis are provided in Tables S1 and S2 at Dryad.

were induced by high temperatures and by osmotic and salinity stresses. *AtHB20* was repressed only by osmotic stress, whereas *AtHB22*, *AtHB51*, and *AtHB52* did not appear to be regulated, according to the applied criterion (Fig. 4; Table S3 at Dryad).

The Root Atlas contained detailed information about single-cell type-specific expression, based on single-cell sequencing data. The observations on the expression of HD-Zip members reported in this repository generally agreed with those from the BAR database, although not always.

From subfamily I, *AtHB1*, *AtHB6*, and *AtHB16* were expressed in all cell types, whereas *AtHB21*, *AtHB22*, *AtHB51*, *AtHB52*, and *AtHB53* were not expressed in any of the analyzed cells, contrary to the information available in the BAR database. Notably, expression data for *AtHB54* were coincident

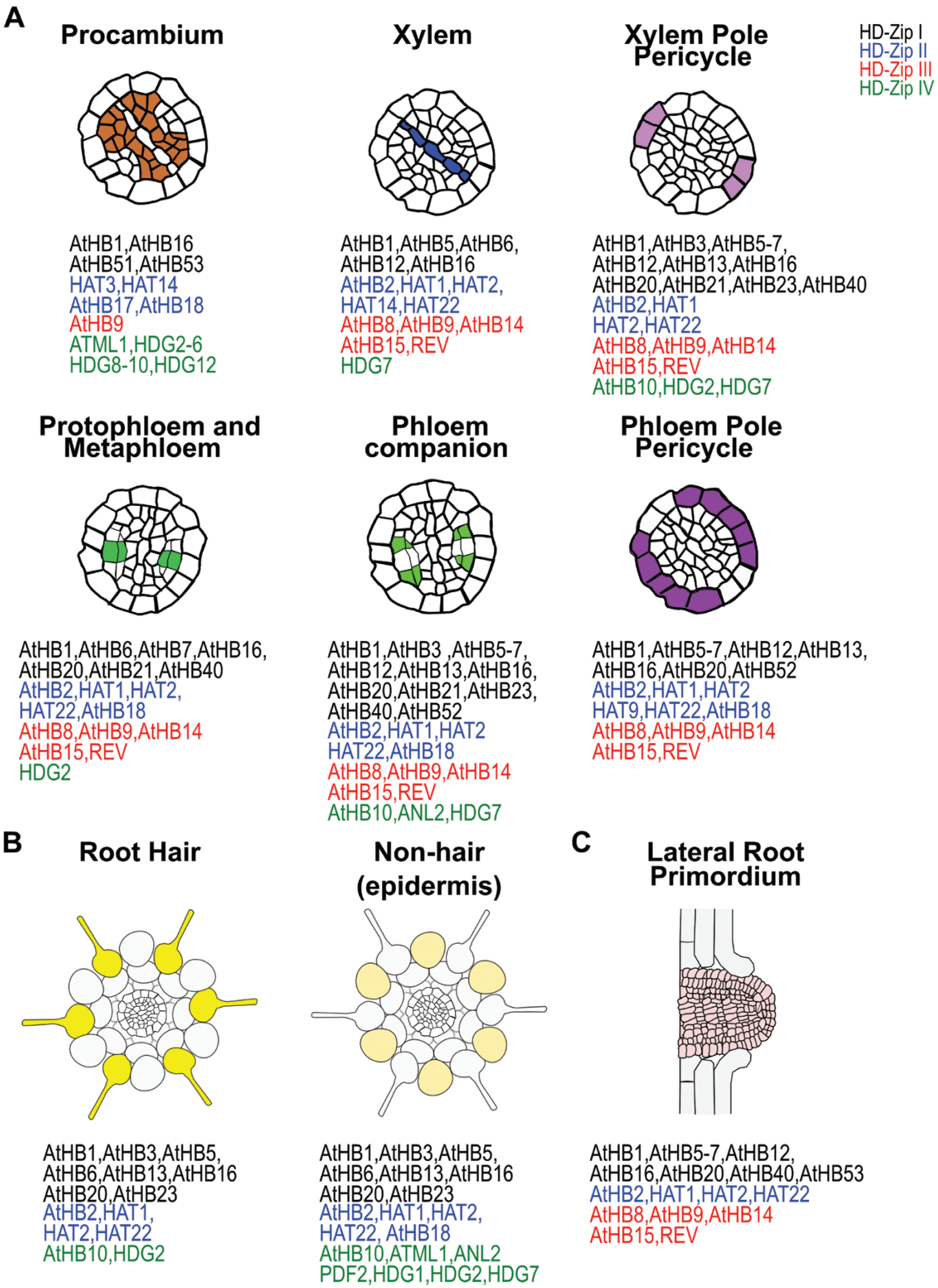

Fig. 3. Genes encoding HD-Zip TFs expressed in the root tip. (A) Drawings of transverse sections indicating different cell types of the central cylinder of the root vasculature: procambium, xylem, xylem-pole pericycle, phloem-pole pericycle, phloem companion, protophloem, and metaphloem. (B) Illustrations of root hair and non-hair (epidermis). (C) Schematic representation of an emerging lateral root primordium. In all panels, members of the HD-Zip family expressed in each tissue are listed and color coded: HD-Zip I (black), HD-Zip II (blue), HD-Zip III (red), HD-Zip IV (green). Key source: BAR from the University of Toronto (http://bar.utoronto.ca/; Brady *et al.*, 2007; Kilian *et al.*, 2007; Dinneny *et al.*, 2008).

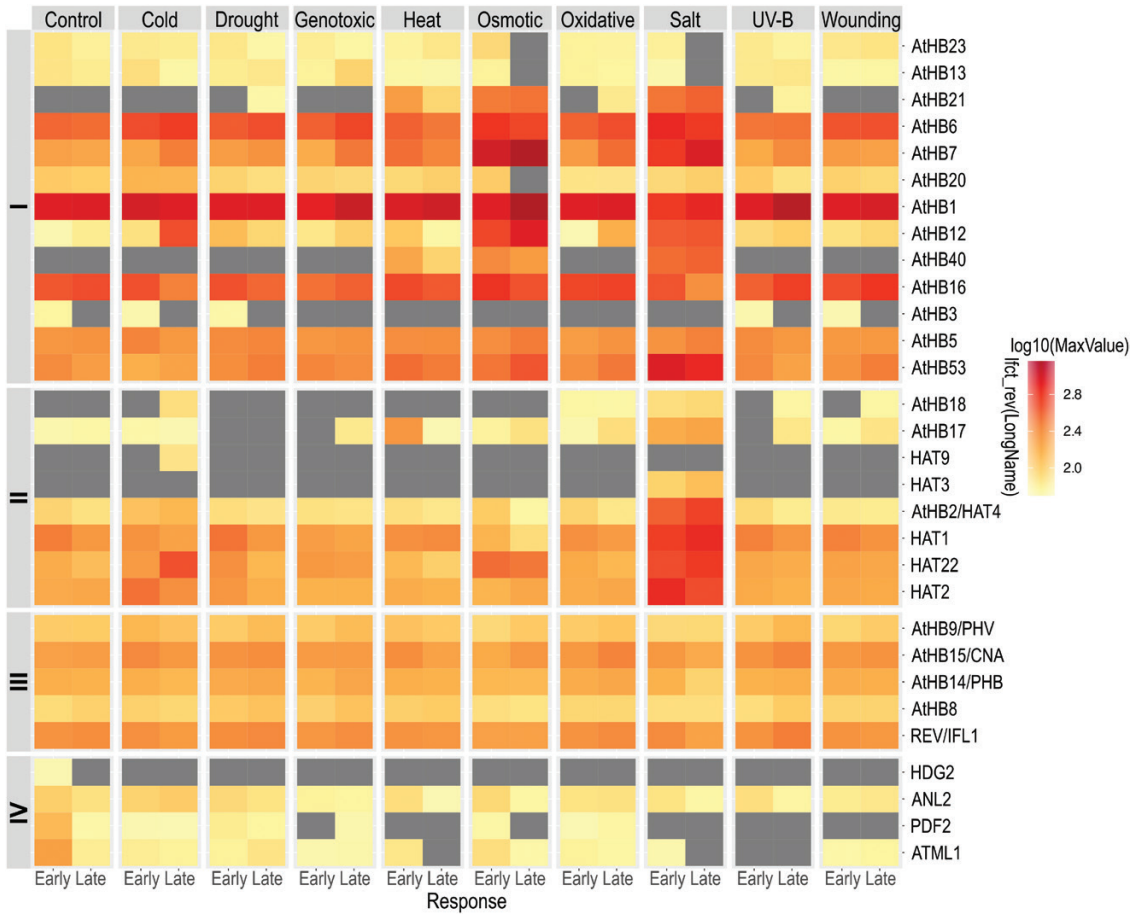

Fig. 4. Abiotic stress factors regulate the expression of HD-Zip TFs. Heat map showing the effect of different environmental stress factors on the expression of genes encoding HD-Zip TFs. The names of the genes are listed on the right. Missing genes are below the expression threshold in all conditions. Treatment is indicated on the upper x-axis and response time to treatment on the lower x-axis, classified as early (15 min, 30 min, 1 h, 3 h, and 4 h) or late (6 h, 12 h, and 24 h) responses. The intensity of color indicates the expression level on a logarithmic scale (right). Data were obtained from the BAR repository. The criterion applied to consider expression is explained in Appendix S1 at Dryad.

between databases, showing an absolute absence of expression. *AtHB7* was expressed in all cell types, except in the epidermis, lateral root, and root hair, whereas its paralog, *AtHB12*, was detected only in the columella, endodermis, and stele. The paralogs *AtHB13* and *AtHB23* were expressed in the same cell types, with only two differences: *AtHB23* was in expressed the stem cell niche and lateral root cap, whereas *AtHB13* was not. *AtHB40* was detected only in the stem cell niche and epidermis, whereas *AtHB5* and *AtHB20* were found in multiple cell types (Fig. 5). Importantly, some apparent discrepancies between the databases could have resulted from the use of roots of different ages for the analyses: the Root Atlas was constructed with data from 10-day-old roots, whereas the experiments collected by the BAR database were conducted with 5- to 7-day-old roots, on average. The two data sources must therefore be considered complementary.

Expression of HD-Zip II members

According to the BAR database, four members (*HAT1*, *HAT2*, *HAT4/AtHB2*, and *HAT22)* were expressed in the lateral root cap and in the columella, and four (*HAT1*, *HAT2*, *HAT4/AtHB2,* and *AtHB17*) were expressed in the QC in normal conditions (Fig. 2A; Table S2 at Dryad). In different zones of the cortex and endodermis, the genes expressed are *HAT1*, *HAT2*, *HAT4/AtHB2*, and *HAT22* (Fig. 2B; Table S2 at Dryad). The same genes were detected in the xylem, the xylem-pole pericycle, the phloem, and the phloem-pole pericycle. In addition to the above-mentioned genes, *HAT14* was expressed in the xylem, and *AtHB18* in the phloem and phloem-pole pericycle. Only *HAT3*, *HAT14*, *AtHB17*, and *AtHB18* were found to be expressed in the procambium (Fig. 3; Table S2, at Dryad). Moreover, several members of subfamily II were detected in the root hair and the non-hair epidermal cells (Fig. 3B; Table S2 at Dryad).

Regarding the effect of abiotic stress factors, *HAT1*, *HAT2*, *HAT3*, *AtHB2/HAT4*, and *HAT22* were strongly induced by sodium chloride, whereas *HAT9* was induced only by cold temperature. *AtHB17* was up-regulated by heat and salinity stresses and down-regulated by drought, genotoxic, and UV-B treatments. *AtHB18* was induced by salinity, cold temperature, UV-B, wounding, and oxidative stresses (Fig. 4; Table S3 at Dryad).

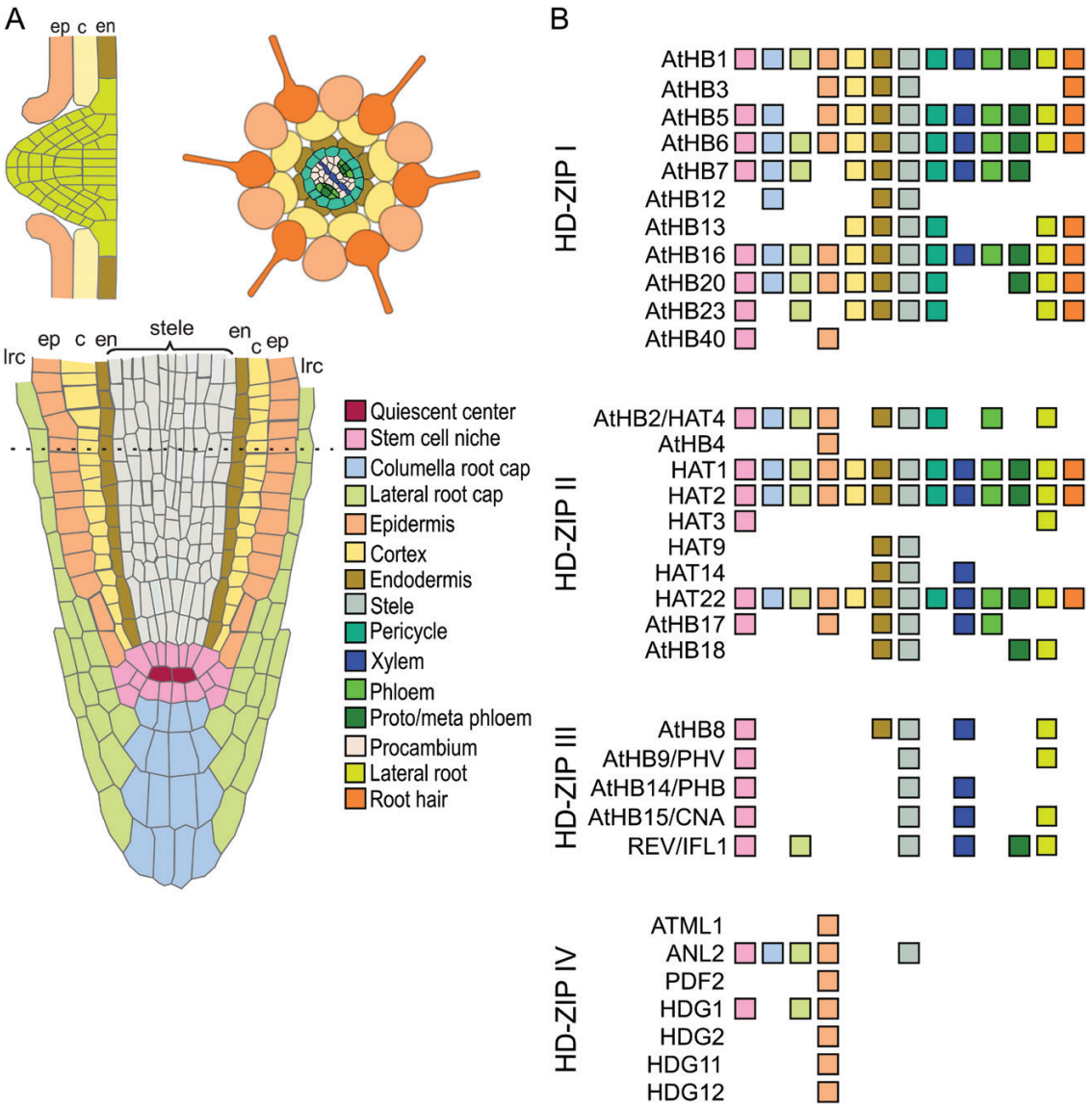

Fig. 5. Expression of HD-Zip TFs according to single-cell transcript analysis. (A) Schematic representation of the cell types constituting an Arabidopsis root. A detail of the lateral root primordium (upper left panel), a transverse section of the root (upper right panel), and a longitudinal section of the root (lower panel) are shown. Different colors indicate each cell type, as indicated in the key. (B) For each gene of the four subfamilies, colored squares indicate the cell types in which the gene is expressed according to the Root Atlas (Zhang *et al.*, 2019; http://wanglab.sippe.ac.cn/rootatlas).

Based on the Root Atlas, *HAT1*, *HAT2*, and *HAT22* are the most widely expressed subfamily II members. Except for *AtHB4* and *HAT3*, all the members of subfamily II were detected in the stele and endodermis. *AtHB4* was found in the epidermis, and *HAT3* was found in the stem cell niche and lateral root. *HAT9* was expressed only in the stele and endodermis (Fig. 5). Notably, for this subfamily, the results obtained from the two databases were substantially different, particularly for the unexpressed members. Such differences are probably due to the "dilution" of single-cell expressed genes in tissue samples containing multiple cells. They could also result from the use of inefficient or cross-hybridizing microarray probes.

Expression of HD-Zip III members

The five members of subfamily III are expressed in specific root cells, but none were expressed in the lateral root cap. *AtHB8*, *AtHB9* (*PHAVOLUTA*), *AtHB14* (*PHABULOSA*), and *IFL1/REVOLUTA/AtHB15* exhibited high expression levels in the columella and QC. *IFL1/REVOLUTA/AtHB15* was also expressed in both the meristematic and the elongation zones of the endodermis, whereas *AtHB14 /PHABULOSA* was expressed only in the meristematic zone of the cortex (Fig. 2; Table S2 at Dryad). None of the subfamily III members were expressed in the root hair or non-hair epidermal cells. The expression of all five members was detected in the xylem,

xylem-pole pericycle, protophloem, metaphloem, phloem companion, phloem-pole pericycle, and lateral root primordium, whereas only *AtHB9* was detected in the procambium (Fig. 3; Table S2 at Dryad). No significant differences were observed in the expression levels of any of these TFs in response to the various abiotic stress treatments (Fig. 4; Table S3 at Dryad).

Based on the Root Atlas, the five members were expressed in the stem cell niche and the stele, and none of them were expressed in the columella root cap, epidermis, or cortex. In the lateral root cap, the only gene expressed was *IFL1/REVOLUTA/AtHB15*, whereas in the endodermis, only *AtHB8* was expressed. All of the members except *AtHB9* were detected in the xylem. A similar scenario was observed in the lateral root, where all subfamily III members were expressed except *AtHB14* (Fig. 5; Table S4 at Dryad).

Expression of HD-Zip IV members

The 16 members of subfamily IV (also called GL2, according to the first identified member, GLABRA2) were expressed in roots in normal growth conditions. Eight HD-Zip IV genes were expressed in the lateral root cap, whereas two were expressed in the QC (*ANL2* and *AtHB10*); only *ANL2* was expressed in the columella (Fig. 2; Table S2 at Dryad). Four members were represented in the procambium, whereas none were expressed in the phloem-pole pericycle (Fig. 3A). Only *HDG7* was found to be expressed in the xylem, while *HDG7*, *ANL2*, and *AtHB10* were expressed in the xylem-pole pericycle and phloem companion. Two members (*AtHB10* and *HDG2*) were detected in root hair, and the same two TFs together with five other members were expressed in the non-hair epidermal cells (Fig. 3B; Table S2 at Dryad). No members of subfamily IV were detected in the lateral root primordium, indicating that this group would not be functional in lateral root development (Fig. 3C; Table S2 at Dryad). The expression of members of this subfamily seemed to be unaffected by external abiotic stimuli. The exceptions were *HDG2*, whose expression was repressed by all the treatments, and *PDF2* and *ATML1*, which were down-regulated by several abiotic stress treatments (Fig. 4; Table S3 at Dryad).

Based on the Root Atlas, seven members of this subfamily were expressed in the epidermis. Only *ANL2* was detected in the stele and the columella, whereas in the stem cell niche and the lateral root cap, both *ANL2* and *HDG1* were expressed (Fig. 5).

What does the literature tell us about HD-Zip expression and functions in roots?

As stated above, functional studies on HD-Zip TFs in roots are scarce. However, during the past few years several papers have revealed crucial functions for these TFs in Arabidopsis roots.

After Arabidopsis, rice is the second species in which HD-Zip members were identified and characterized. Twelve members of subfamily IV were identified and phylogenetic trees including these rice proteins were constructed (Schrick *et al.* 2004; Nakamura *et al.*, 2006). Later, and based on a previous description of seven members belonging to subfamilies I, II, and III (Meijer *et al.*, 1997, 2000), Agalou *et al.* (2008) identified 33 proteins from these three subfamilies. Among them, 14 were from subfamily I, 14 from subfamily II, and five from subfamily III. The expression of many of these genes was detected in roots.

The number of members of each subfamily varies with respect to those in Arabidopsis, not only in rice but also in other plants in which these genes have been identified. However, in general, subfamily I is usually the largest, followed by subfamilies IV and II, with subfamily III being the smallest.

In soybean, 101 HD-Zip genes were identified; among them, 88 existed as whole-genome duplication-derived gene pairs and 20 were differentially expressed in roots after abiotic stress treatments (water deficit and salinity), as revealed by transcriptome analysis (Belamkar *et al.*, 2014). Among the identified soybean genes, 36 belonged to subfamily I, 24 to subfamily II, 11 to subfamily III, and 30 to subfamily IV. Belamkar *et al.* (2014) did a complete phylogenetic analysis, improving on previous ones, and also conducted a survey of the expression of these genes in 17 different tissues under 24 conditions. However, the functions of these genes were suggested by relating them to sequence similarity and expression patterns of Arabidopsis HD-Zip members (Belamkar *et al.*, 2014). Unfortunately, no functional experiments have been reported so far.

In maize, 55 HD-Zip genes were identified and found to belong to the four subfamilies: 17 from subfamily I and five from group III, as in Arabidopsis, 18 from group II, and 15 from group IV (Zhao *et al.*, 2011). The expression of these genes was analyzed before and after water deficit but not specifically in roots.

Arabidopsis HD-Zip subfamily I members

AtHB6 is expressed in the root tip of 3-day-old seedlings; this expression was observed by histochemistry in transgenic plants carrying the reporter gene β-*glucuronidase* (*GUS*) with expression being driven by the *AtHB6* promoter (Söderman *et al.*, 1999). Expression in seedlings was induced by water deficit, osmotic stress, or exogenous treatment with abscisic acid (ABA). However, this induction was not significant in a culture of root cells. The authors suggested that the TF AtHB6 may have a role in the control of cell division and/or differentiation (Söderman *et al.*, 1999). Analysis of the *AtHB6* promoter directing GUS expression in transgenic plants supported the assigned function. In these plants, GUS activity was detected in the cell division zone of the primary and lateral roots and in zones of cell differentiation (Fig. 6).

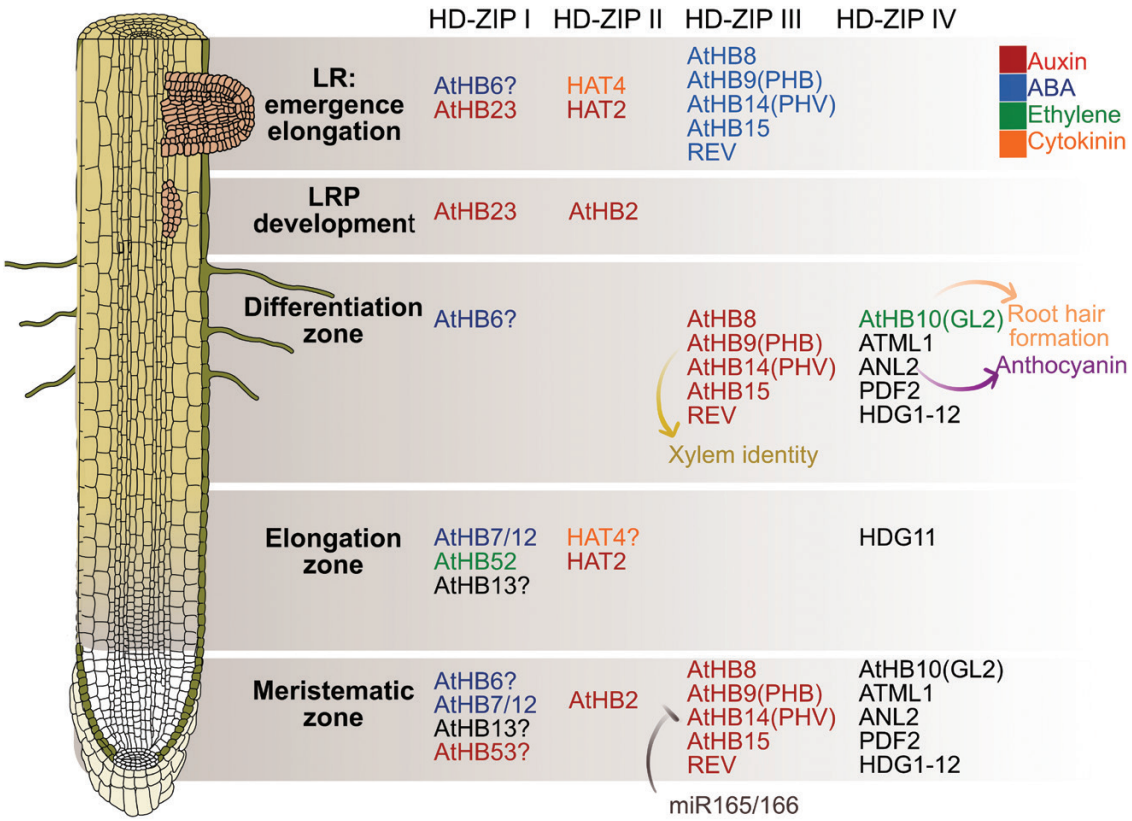

Fig. 6. HD-Zip TFs have specific roles depending on their pattern of expression in the primary root and lateral root. Schematic representation of the main root, lateral root primordium (LRP), and lateral root (LR), indicating the different zones. The function of HD-Zip genes from the four classes, according to the literature, is indicated on the right. Hormone-regulated genes are indicated with different colors: auxin (red), ABA (blue), ethylene (green), cytokinin (orange). Question marks denote genes that putatively act in the indicated zone.

AtHB12 expression was detected in roots of 14- and 23-day-old plants but not in 45-day-old plants (Ré *et al.*, 2014), whereas its paralog *AtHB7* was absent in early-stage roots. However, expression in older roots cannot be ruled out because *AtHB7* is expressed in advanced developmental stages, in which the roots were not analyzed (Ré *et al.*, 2014). Remarkably, double mutant *athb7/athb12* plants, but neither of the single mutants, exhibited shorter roots than the wild-type control. Plants overexpressing *AtHB7* exhibited a differential root phenotype, probably as a result of ectopic expression or from the regulation of *AtHB12*, which was demonstrated (Ré *et al.* (2014). Both genes were induced by abiotic stress factors, including water deficit and salinity, and also by ABA treatment. More recently, this pair of genes was reported to be up-regulated by aluminum stress in the roots, particularly in the transition zone (Liu *et al.*, 2020). In that report, the authors showed that *athb7* mutant plants have a smaller than normal root meristem zone, due to the presence of fewer cells in this zone, while in the elongation zone, the cortical cells are shorter than those of the wild type (Fig. 6). When seedlings were subjected to aluminum stress, these genes displayed an antagonistic role in root elongation; *AtHB7* promoted resistance to aluminum stress, whereas *AtHB12* had the opposite effect (Liu *et al.*, 2020).

A predictive co-expression network study identified *AtHB13* as a crucial player in the seed-to-seedling phase transition (Silva *et al.*, 2016). AtHB13 was involved in late seedling establishment, and *athb13* mutants exhibited longer primary roots compared with controls, indicating that AtHB13 is a negative regulator of early root growth, probably through the inhibition of cell division or cell elongation (Fig. 6).

The paralog of *AtHB13*, *AtHB23*, was functionally characterized. This gene is expressed in lateral root primordia, inhibiting lateral root initiation (Fig. 6), and it was shown to be the link between ARF7/19 (Auxin Response Factors) and LAX3. More precisely, *AtHB23*-silenced plants showed more initiated roots than controls. It was found that ATHB23 targeted the auxin influx carrier gene *LAX3*, which was induced, and *LBD16*, belonging to the Lateral Organ Boundaries family, which was repressed. Notably, the study of this gene revealed that molecular programs for higher-order roots differ from those leading to lateral roots from the main root, given that the expression patterns of both *AtHB23* and *LBD16* significantly differed between these two processes (Perotti *et al.*, 2019, 2020).

AtHB52 was described as a key player in the crosstalk between auxin and ethylene signaling, modulating auxin transport downstream of *EIN3* (Miao *et al.*, 2018). The expression of this gene was regulated by ethylene, and *athb52* mutants were insensitive to this hormone. Moreover, both mutants and overexpressors showed distorted auxin distribution and gravitropism. Target genes of the AtHB52 TF were identified by binding experiments both *in vitro* and *in vivo*. Among these targets, *PIN2*, *WAVY ROOT GROWTH1* (*WAG1*), and *WAG2*, seemed to be involved in ethylene-mediated inhibition of root elongation (Miao *et al.*, 2018) (Fig. 6). Finally, *AtHB53* was shown to be expressed in the root meristem by whole-mount *in situ* hybridization and non-quantitative RT–PCR, and was assigned a role in auxin/cytokinin signaling pathways in roots (Son *et al.*, 2005) (Fig. 6).

Subfamily I members in other plant species

PuHox52 from *Populus ussuriensis* belongs to the γ/I clade (Henriksson *et al.*, 2005; Arce *et al.*, 2011), like *AtHB7* and *AtHB12* in Arabidopsis. Its expression was detected at the basal ends of stems by cutting (Wei *et al.*, 2020). Its overexpression (in the same species) significantly increased the number of adventitious roots and shortened the rooting time, whereas its suppression led to the opposite phenotype. Several target genes of this TF were identified using a multilayered hierarchical gene regulatory network, among them 15 TFs. Notably, these TFs included an HD-Zip II putative homolog, HAT2 (Wei *et al.*, 2020).

In rice, *OsHOX4* is expressed in the roots and repressed by drought stress. Loss-of-function plants, generated by RNAi, did not show a differential phenotype, whereas overexpressors exhibited severe developmental defects. Analysis of the stems and leaves of *OsHOX4*-overexpressing plants suggested a role for OsHOX4 in stem elongation, maturation, and senescence. However, these putative functions were not confirmed with loss-of-function plants, probably due to compensation by its paralog, *OsHOX20* (Agalou *et al.*, 2008).

The sunflower divergent member *HaHB4* was described as being weakly expressed in roots but strongly induced in this organ by drought (Gago *et al.*, 2002). The promoter of this gene was characterized by transforming Arabidopsis plants with a construct in which the *HaHB4* promoter drove the expression of the *GUS* reporter gene. The expression, observed by histochemistry, was found to spread throughout the whole root, including lateral roots. The mutation of specific *cis*-acting elements limited the expression to the lateral root primordium, allowing the identification of specific root elements in the promoter (Manavella *et al.*, 2008). This gene conferred drought tolerance on various plant species, including Arabidopsis, soybean, and wheat (Dezar *et al.*, 2005; González *et al.*, 2019; Ribichich *et al.*, 2020). However, its function in roots has not yet been uncovered.

Another divergent subfamily I member, *HaHB11*, is expressed in roots. When it was overexpressed in Arabidopsis, the transgenic plants had significantly longer primary roots. The expression of the gene was visualized along the main root, in the lateral root primordium, and later in the secondary roots (Cabello *et al.*, 2016, 2017).

In *Medicago truncatula*, *MtHB1*, which exhibits high sequence similarity with the Arabidopsis subfamily I members *AtHB7* and *AtHB12*, is expressed in primary and lateral root meristems and is induced by salt stress (Ariel *et al.*, 2010*b*). Its constitutive expression affected the architecture of *Medicago* roots, and the mutant exhibited enhanced root emergence. This gene was found to directly repress the expression of the LOB family TF gene *LBD1*, conferring salinity tolerance (Ariel *et al.*, 2010*a*).

Arabidopsis HD-Zip subfamily II members

Transcription of *AtHB2/HAT4* is induced by changes in the red:far-red light ratio, promoting shade avoidance. Increased levels of this gene's transcripts inhibited cell proliferation in the main root and lateral root formation, whereas treatment with auxin rescued this phenotype. Reduction in the *AtHB2/HAT4* transcript level led to the opposite phenotype (Steindler *et al.*, 1999). Transgenic plants overexpressing this gene, as well as plants expressing it after chemical induction, exhibited abnormal root gravitropism, a typical auxin-related phenotype. In seedlings in which *AtHB2* expression was chemically induced, the transcript levels of some auxin biosynthetic and transport genes were reduced, indicating a role for this TF in auxin patterning (He *et al.*, 2020).

According to a microarray analysis, *AtHB2/HAT4* was described as induced by cytokinin in 5-day-old seedlings (Brenner *et al.*, 2005). Plants overexpressing *HAT4* exhibited shorter roots with less branching, whereas the *hat4* mutant showed the opposite phenotype, both compared with controls. In view of the characteristics of *HAT4* mutants and overexpressors, and considering that the expression of this gene is up-regulated by cytokinin, the authors suggested a cytokinin-associated role for *HAT4* in roots (Köllmer *et al.*, 2011).

Interestingly, and although the classification of the HD-Zip TFs into four subfamilies is based on markedly different structural features, members from subfamilies II and III interact in auxin-dependent plant development. Loss-of-function HD-Zip II mutations were found to directly affect auxin distribution and responses, and HD-Zip III TFs regulated the expression of subgroup γ and δ genes (Turchi *et al.*, 2015).

Transcriptome analysis comparing plants treated with indole-3-acetic acid (IAA) for 15 min and untreated plants revealed that *HAT2* is one of the 29 genes induced soon after this treatment. Notably, this gene was not responsive to other hormones. *HAT2*-overexpressing plants showed reduced lateral root elongation and sensitivity to auxin, compared with controls. Remarkably, this gene played opposite roles in shoot and root tissues, regulating auxin-mediated morphogenesis

(Sawa *et al.*, 2002). It is important to note that in *HAT2*-overexpressing plants, the transcript levels of the other subfamily II members were regulated. More precisely, *AtHB2*, *AtHB4*, *HAT1*, *HAT3*, *HAT9*, and *HAT22* were strongly repressed, whereas the expression of members of other subfamilies was not altered (Sawa *et al.*, 2002). In addition to the interplay between HD-Zip II proteins, this assay indicated that the morphologic effect observed in roots could be the result of these repressed genes.

Subfamily II members in other plant species

In common wheat, 113 HD-Zip members have been identified; among them, 32 belong to subfamily II (Li *et al.*, 2020*a*). The canonical auxin-responsive element TGTCTC is overrepresented in the promoters of wheat HD-Zip II-encoding genes. In roots, the expression of *TaHDZ19-3A/3B/3D*, *TaHDZ20-1A/1B/1D*, and *TaHDZ21-2A/2B/2D* was strongly induced by auxin, whereas *TaHDZ21-23-7A/7D* was repressed after the same treatment, suggesting a role for this gene in the auxin-related pathway similar to that of its Arabidopsis homolog *AtHB2* (He *et al.*, 2020). Moreover, other members of subfamily II in Arabidopsis are involved in auxin distribution and responses (Turchi *et al.*, 2013, 2015). In the case of wheat subfamily II members, a role in auxin distribution and response has not been experimentally corroborated so far, and more data will be necessary to confirm orthology.

The sunflower gene *HaHB10* has high sequence similarity with *AtHB2/HAT4*, and it is expressed in different organs, including roots at different developmental stages. This gene is regulated by the quality and intensity of light and is involved in flowering. Functional studies in roots have not yet been carried out, but the functions of this gene could be related to those of its putative homolog in Arabidopsis (Dezar *et al.*, 2011).

Arabidopsis HD-Zip subfamily III members

Members of subfamily III were found to be associated with root development 15 years ago. Generation of overexpressors and loss-of-function mutants, particularly in the genes *REVOLUTA* (*REV*), *PHABULOSA* (*PHB*), and *PHAVOLUTA* (*PHV*), and in *KANADI* genes, revealed that the TFs encoded by these genes play crucial roles in the ontogeny of lateral roots. *KANADI* genes belong to the GARP TF family and have been described as regulators of organ polarity. They are required for abaxial identity in both leaves and carpels. They are also required for the proper regulation of auxin flow in early embryogenesis (Eshed *et al.*, 2001, 2004). Hawker and Bowman (2004) demonstrated that the presence of HD-Zip III TFs susceptible to auxin induction was required for meristematic activity in the pericycle (Fig. 6). In that study, assays were carried out on the Ler genotype, whereas most subsequent experiments described in other works were done with Col-0 plants. It is important to note this difference because root development has been shown to follow different programs in these two genotypes (Perotti *et al.*, 2020).

PHABULOSA (PHB) and PHAVOLUTA (PHV) must be confined in the apical meristem to enable correct establishment of the root meristem. When the regulation of PHB and PHV expression by a microRNA (miRNA)-dependent pathway failed, the precise elaboration of the embryonic root development program was prevented and resulted in an embryo lethal phenotype (Grigg *et al.*, 2009).

miRNAs are small RNAs that play crucial roles in the regulation of gene expression. They are involved in multiple plant processes and are required for normal growth, negatively regulating TFs and hormone receptors. In roots, they are involved in numerous developmental processes, such as vascular differentiation, root apical meristem functioning, and stress responses. They are organized in multigenic families, and among them, the family miR165/166 targets members of the subfamily III HD-Zip genes (Couzigou and Combier, 2016).

Plants with mutations in genes encoding HD-Zip III TFs lose their xylem cells, indicating that these genes are essential for xylem identity (Carlsbecker *et al.*, 2010). In the stele, HD-Zip III members determine the xylem pattern. High or low expression levels of HD-Zip III TFs define the formation of metaxylem or protoxylem, respectively. Moreover, these genes are regulated by miR165/166; the overexpression of these miRNAs repressed the expression of HD-Zip III proteins, inducing root growth by enhancing cell division and meristematic activity, while the opposite effect was observed when HD-Zip III genes were overexpressed (Singh et al., 2014). Moreover, ABA induced the expression of miR165a/166b (a and b denote particular genes within each miRNA gene family) and repressed *ARGONAUTE10* (*AGO10/AT5G43810*, a principal component of the RNA-induced silencing complex), which is an miR165a/166b repressor, which resulted in repression of the expression of all five HD-Zip III members. A similar regulatory event occurs to maintain the shoot apical meristem: *ago10* mutants exhibit abnormally high levels of miR165/166, leading to a reduction of HD-Zip III transcripts (Liu *et al.*, 2009). In this way, ABA regulates xylem pattern and maturation. Specifically, it inhibits the reduction of the QC, specifically at the pre-emergence stage, and also reduces the expression of lateral root-regulating HD-Zip III proteins through the action of miRNAs (Bloch *et al.*, 2019; Fig. 6). Interestingly, besides the regulation by miRNAs, HD-Zip III TFs are also regulated by LITTLE ZIPPER (ZPR) proteins, which possess a LZ but lack the HD. ZPR proteins were shown to heterodimerize *in vitro* and *in vivo* with HD-Zip III proteins, preventing them from binding to DNA and, in consequence, functionally inhibiting them (Wenkel *et al.*, 2007).

HD-Zip III TFs modulate the expression of genes encoding core auxin response molecules. PHB directly regulates *MONOPTEROS/AUXIN RESPONSE FACTOR5*, which encodes a key TF in vascular formation, and *IAA20*, which encodes an auxin/IAA protein that can interact with

MONOPTEROS. MONOPTEROS would cooperate with PHB in activating *IAA20*, while IAA20 would repress *MONOPTEROS*, forming a complex regulatory network that ultimately results in continuous xylem development despite perturbations in auxin levels (Müller *et al.* 2016).

The expression of HD-Zip III TFs is up-regulated by auxin, and once induced they promote xylem identity and quiescence of the organizer cells. This is supported by the study of a quadruple mutant lacking four HD-Zip III genes, which shows patterning defects and reduced xylem formation in the primary root vasculature. The inducible overexpression of *AtHB8* (resistant to miR164 and miR166) inhibited cell division in the stem cells, indicating the role of these TFs in promoting cellular quiescence (Smetana *et al.*, 2019; Fig. 6).

Both HD-Zip III members and their regulating miR166/165 have been shown to be regulated by other hormones aside from auxin and ABA. In this way, these proteins mediate root development, both transcriptionally through phytohormones and *KANADI* genes, and post-transcriptionally via miRNAs (Singh *et al.*, 2017). Gibberellin, cytokinin, jasmonic acid, and salicylic acid induced miRNA expression at different time points. This regulation exhibited a time-dependent behavior; for example, treatment with salicylic acid induced the miRNAs during a period of 12 h but inhibited them after 24 h. A complex model involving miRNAs, phytohormones, HD-Zip III TFs, and *KANADI* genes, leading to the repression or induction of root growth, was recently proposed by Singh *et al.* (2017).

Subfamily III members in other plant species

In soybean, 12 putative HD-Zip III genes have been identified, including *GmHD-ZIP III-1* to *GmHD-ZIP III-12*. The encoded proteins are regulated by other TFs, specifically, the members of the ZPR family. Using yeast two-hybrid assays, GmZPR3d was discovered to interact with GmHD-Zip III-1 (a potential ortholog of PHB and PHV) and GmHD-Zip III-2 (a potential ortholog of ATHB15), showing co-expression in the root and parenchyma tissue of the nodule. These interactions probably prevent DNA binding by GmHD-Zip III proteins, since ZPR does not possess an HD. Overexpression of *GmZPR3d* in soybean resulted in large nodules with a larger central zone, indicating a role for this pair of interacting proteins in nodule development in this species (Damodaran *et al.*, 2019).

In Tibetan wild barley, *HvHOX9* was identified as a target gene of miR166b and was significantly induced in the root tip only under aluminum stress. Its closest ortholog is wheat *TaHOX9*. Virus-induced silencing of HvHOX9 enhanced sensitivity to aluminum but not to other metals or to low pH. Moreover, these silenced plants accumulated more aluminum in the root cell wall after aluminum exposure, but the concentration of aluminum in the root cap was not altered. HvHOX9, and probably other HD-Zip III proteins, were thus suggested to be involved in aluminum tolerance in barley (Feng *et al.*, 2020).

The rice subfamily III gene *OsHB4* (*Os03g43930*) is, like the other members of this subfamily, a target of miR166. The expression of this gene is responsive to the presence of toxic cadmium. Overexpressor *35S:OsHB4* lines had significantly lower cadmium concentrations in the roots than wild-type plants, and the opposite scenario was observed in shoots, suggesting that OsHB4 plays a role opposite to that of miR166 in promoting cadmium translocation from roots to shoots (Ding *et al.*, 2018).

Arabidopsis HD-Zip subfamily IV members

The roles and expression patterns of HD-Zip IV genes have been reviewed by Chew *et al.* (2013). Many of these genes are specifically or preferentially expressed in plant epidermal or subepidermal cells and are involved in the regulation of lipid biosynthesis and transport. In roots, HD-GLABRA2 (HD-GL2) was shown to be a negative regulator in phospholipid signaling involving the START domain (Ohashi *et al.*, 2003).

HDGTFs act antagonistically with AIL (AINTEGUMENTA-LIKE) to control cell proliferation (Horstman *et al.*, 2015), and HDG1, HDG11, and HDG12 interact with BBM (BABY BOOM, belonging to the AP2/ERF family). Overexpression of the HD-Zip IV proteins provokes an arrest in the root meristem that restricts root growth. Reduced levels of expression of these genes promote cell differentiation. Overexpression of *HDG1* promotes giant cell identity, whereas *hdg1* mutants do not show an obvious root meristem phenotype. The interaction between HDG and AIL occurs through protein–protein interactions and also at the transcriptional level; these TFs regulate each other and share target genes (Horstman *et al.*, 2015).

HDG11 was reported to participate in drought tolerance involving auxin. In Arabidopsis, this TF regulates *IAA28* (Rogg *et al.*, 2001), and its mutation led to a down-regulation of the latter gene and a subsequent drought-sensitive phenotype in roots. In contrast, overexpression of *HDG11* resulted in drought tolerance by enhancing root architecture and stomatal density (Rogg *et al.*, 2001; Yu *et al.*, 2008). This Arabidopsis gene, also called *AtEDT1* (*ENHANCED DROUGHT TOLERANCE1/HOMEODOMAIN GLABROUS11*), has been ectopically overexpressed in other species, conferring similar features in *Oryza sativa*, *Ipomea batatas*, and *M. truncatula* (Ruan *et al.*, 2012; Yu *et al.*, 2013; Zheng *et al.*, 2017). The results of yeast-one hybrid and chromatin immunoprecipitation assays indicated that HDG11 positively regulates several cell-wall-loosening protein genes (Xu *et al.*, 2014).

GLABRA2/AtHB10 was first associated with trichomes. Later, it was shown that its expression is not unique to trichome tissues, and it is also expressed in the roots in Arabidopsis. The characterization of mutants of this gene (*gl2-1*) allowed determination that it is necessary for root hair development in a subset of epidermal cells. This process is regulated by ethylene

(Di Cristina *et al.*, 1996). Moreover, *gl2* mutant plants exhibited root hair development from hairless epidermal cells, although other events taking place during root hair differentiation were not affected (Masucci *et al.*, 1996). Moreover, it was shown that GLABRA2 recognizes and binds the promoter of *VLN1* (*Actin-Bundling Protein Villin1*), regulating the expression of this target gene specifically in root hairs, particularly in response to osmotic stress. The overexpression of *VLN1* suppressed the *gl2* mutant phenotype in root hair growth and actin dynamics (Wang *et al.*, 2020).

ANL2 (*ANTHOCYANINLESS2*) is involved in the accumulation of anthocyanin in roots. This accumulation was strongly repressed in subepidermal cells of *anl2* mutant plants, but it was reduced only slightly in epidermal cells of the Ler ecotype. The mutants showed aberrant cellular organization in the primary root (Kubo *et al.*, 1999).

Subfamily IV members in other plant species

In maize, ANL2 was shown to control the cellular organization of the primary root (Javelle *et al.*, 2011). Moreover, transcripts corresponding to *ZmOCL1* (OCL for outer cell layer), belonging to the HDGL1 subclass, were detected in early embryos, prior to protoderm development, also in maize. Expression of *ZmOCL1* was then detected in the L1 cell layer of the primary root, suggesting that this gene is involved in the organization of the primary root primordium (Ingram *et al.*, 1999).

The cotton (*Gossypium arboretum*) subfamily IV members *GaHOX1* and *GaHOX2* were able to rescue the *gl2* mutant trichome phenotype. Since these genes are putative orthologs of GLABRA2, similar functions in roots could be assigned, although only the trichome phenotype was experimentally verified (Guan *et al.*, 2008).

Among the 15 genes encoding HD-Zip IV TFs in tobacco, only *NtHD-ZIP-IV-15* has been detected in the root epidermis. Its expression was induced by multiple abiotic stress factors, indicating a functional role in the root epidermis in response to environmental conditions (Zhang *et al.*, 2019).

The gene *HaHR1* (*Helianthus annuus Homeobox Root 1*) was isolated from a sunflower cDNA library. Its expression in roots at different developmental stages was confirmed by Northern blot and RT–PCR (Valle *et al.*, 1997). The function of this gene in roots was inferred from its high sequence similarity with GLABRA2.

Secondary and higher-order roots

The modulation of root architecture allows plants to adapt to environmental conditions spatially and temporally. Compared with the primary root, lateral roots constitute most of the total root system; such a system is established by each order of roots emerging from a previous one. Studies on higher-order roots are significantly rarer, in fact almost non-existent, compared with studies on the main and primary lateral roots. This interesting subject is unexplored, and the knowledge to be gained could have a substantial impact on crop improvement. Future studies will almost certainly focus on higher-order root development due to its importance in plant adaptation to inhospitable soils. Summarizing the little knowledge that exists about HD-Zip TFs in this developmental event, we can note that AtHB23 was recently described as having different target genes in root initiation from primary, compared with secondary, roots (Perotti *et al.*, 2019, 2020).

Conclusion

The fine regulation of root development is crucial for plant growth and survival in any external conditions. Other than a few exceptions, mostly some members of subfamilies III and IV, HD-Zip TFs have not been described as functionally associated with root development. The fact that HD-Zip TF genes exhibit very specific expression patterns in particular root cell types, exceeding the transcript levels of marker genes, tempts us to speculate that they have functions in root development. Here, we have shown that for HD-Zip TFs, information available in the relevant databases has enormous value. By carefully analyzing this information, we revealed which genes are expressed in each cell type and under a wide range of abiotic stress conditions, even though no functional reports are available in many cases. However, such data arise from particular experiments carried out with plants of different ages and growth conditions. Furthermore, discrepancies between the databases resulted from the materials and techniques used—that is, single cells versus tissues, and microarrays versus next-generation sequencing. Although functional characterization is still needed to understand the role of particular genes in root development, data held in the repositories helped us to support not only the importance of HD-Zip TFs but also the differences between the subfamilies in their putative or confirmed roles, as well as the regulation of these genes by different external conditions.

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Author contributions

MFP and RLC conceived and designed the work; ALA performed the quantitative analyses on data obtained from repositories; RLC wrote the manuscript with contributions from MFP; MFP revised

the manuscript and prepared the illustrations; all authors approved the manuscript for publication.

Conflict of interest

The authors declare no competing interests.

Data availability

Methods used in data analysis obtained from the BAR and Root Atlas repositories, primary expression of Arabidopsis root genes encoding HD-Zip TFs, and statistical analysis of the expression data are available at Dryad Digital Repository. https://doi.org/10.5061/dryad.mpg4f4qzb; Perotti *et al*. (2021).

References

Agalou A, Purwantomo S, Overnäs E, *et al.* 2008. A genome-wide survey of HD-Zip genes in rice and analysis of drought-responsive family members. Plant Molecular Biology **66**, 87–103.

Arce AL, Raineri J, Capella M, Cabello JV, Chan RL. 2011. Uncharacterized conserved motifs outside the HD-Zip domain in HD-Zip subfamily I transcription factors; a potential source of functional diversity. BMC Plant Biology **11**, 42.

Ariel FD, Diet A, Crespi M, Chan RL. 2010a. The LOB-like transcription factor MtLBD1 controls *Medicago truncatula* root architecture under salt stress. Plant Signaling and Behavior **5**, 1666–1668.

Ariel F, Diet A, Verdenaud M, Gruber V, Frugier F, Chan R, Crespi M. 2010b. Environmental regulation of lateral root emergence in *Medicago truncatula* requires the HD-Zip I transcription factor HB1. The Plant Cell **22**, 2171–2183.

Ariel FD, Manavella PA, Dezar CA, Chan RL. 2007. The true story of the HD-Zip family. Trends in Plant Science **12**, 419–426.

Atkinson JA, Rasmussen A, Traini R, Voß U, Sturrock C, Mooney SJ, Wells DM, Bennett MJ. 2014. Branching out in roots: uncovering form, function, and regulation. Plant Physiology **166**, 538–550.

Banda J, Bellande K, von Wangenheim D, Goh T, Guyomarc'h S, Laplaze L, Bennett MJ. 2019. Lateral root formation in *Arabidopsis*: a well-ordered LRexit. Trends in Plant Science **24**, 826–839.

Belamkar V, Weeks NT, Bharti AK, Farmer AD, Graham MA, Cannon SB. 2014. Comprehensive characterization and RNA-Seq profiling of the HD-Zip transcription factor family in soybean (*Glycine max*) during dehydration and salt stress. BMC Genomics **15**, 950.

Bloch D, Puli MR, Mosquna A, Yalovsky S. 2019. Abiotic stress modulates root patterning via ABA-regulated microRNA expression in the endodermis initials. Development **146**, dev177097.

Brady SM, Orlando DA, Lee JY, Wang JY, Koch J, Dinneny JR, Mace D, Ohler U, Benfey PN. 2007. A high-resolution root spatiotemporal map reveals dominant expression patterns. Science **318**, 801–806.

Brenner WG, Romanov GA, Köllmer I, Bürkle L, Schmülling T. 2005. Immediate-early and delayed cytokinin response genes of *Arabidopsis thaliana* identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades. The Plant Journal **44**, 314–333.

Bürglin TR. 2011. Homeodomain subtypes and functional diversity. SubCellular Biochemistry **52**, 95–122.

Cabello JV, Giacomelli JI, Piattoni CV, Iglesias AA, Chan RL. 2016. The sunflower transcription factor HaHB11 improves yield, biomass and tolerance to flooding in transgenic Arabidopsis plants. Journal of Biotechnology **222**, 73–83.

Cabello JV, Giacomelli JI, Gómez MC, Chan RL. 2017. The sunflower transcription factor HaHB11 confers tolerance to water deficit and salinity to transgenic Arabidopsis and alfalfa plants. Journal of Biotechnology **257**, 35–46.

Capella M, Ribone PA, Arce AL, Chan RL. 2016. Homeodomain-leucine zipper transcription factors: structural features of these proteins, unique to plants. In: Gonzalez DH, ed. Plant transcription factors: evolutionary, structural and functional aspects. London: Academic Press, 113–126.

Carlsbecker A, Lee JY, Roberts CJ, *et al.* 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. Nature **465**, 316–321.

Chew W, Hrmova M, Lopato S. 2013. Role of homeodomain leucine zipper (HD-Zip) IV transcription factors in plant development and plant protection from deleterious environmental factors. International Journal of Molecular Sciences **14**, 8122–8147.

Ciarbelli AR, Ciolfi A, Salvucci S, Ruzza V, Possenti M, Carabelli M, Fruscalzo A, Sessa G, Morelli G, Ruberti I. 2008. The Arabidopsis homeodomain-leucine zipper II gene family: diversity and redundancy. Plant Molecular Biology **68**, 465–478.

Clowes FAL. 1958. Development of quiescent centres in root meristems. New Phytologist **57**, 85–88.

Couzigou JM, Combier JP. 2016. Plant microRNAs: key regulators of root architecture and biotic interactions. New Phytologist **212**, 22–35.

Damodaran S, Dubois A, Xie J, Ma Q, Hindié V, Subramanian S. 2019. GmZPR3d interacts with GmHD-ZIP III proteins and regulates soybean root and nodule vascular development. International Journal of Molecular Sciences **20**, 827.

de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X. 2007. Root system architecture: opportunities and constraints for genetic improvement of crops. Trends in Plant Science **12**, 474–481.

Dezar CA, Gago GM, Gonzalez DH, Chan RL. 2005. *Hahb-4*, a sunflower homeobox-leucine zipper gene, is a developmental regulator and confers drought tolerance to *Arabidopsis thaliana* plants. Transgenic Research **14**, 429–440.

Dezar CA, Giacomelli JI, Manavella PA, Ré DA, Alves-Ferreira M, Baldwin IT, Bonaventure G, Chan RL. 2011. HAHB10, a sunflower HD-Zip II transcription factor, participates in the induction of flowering and in the control of phytohormone-mediated responses to biotic stress. Journal of Experimental Botany **62**, 1061–1076.

Di Cristina M, Sessa G, Dolan L, Linstead P, Baima S, Ruberti I, Morelli G. 1996. The *Arabidopsis* Athb-10 (GLABRA2) is an HD-Zip protein required for regulation of root hair development. The Plant Journal **10**, 393–402.

Ding Y, Gong S, Wang Y, Wang F, Bao H, Sun J, Cai C, Yi K, Chen Z, Zhu C. 2018. MicroRNA166 modulates cadmium tolerance and accumulation in rice. Plant Physiology **177**, 1691–1703.

Dinneny JR, Long TA, Wang JY, Jung JW, Mace D, Pointer S, Barron C, Brady SM, Schiefelbein J, Benfey PN. 2008. Cell identity mediates the response of *Arabidopsis* roots to abiotic stress. Science **320**, 942–945.

Eshed Y, Baum SF, Perea JV, Bowman JL. 2001. Establishment of polarity in lateral organs of plants. Current Biology **11**, 1251–1260.

Eshed Y, Izhaki A, Baum SF, Floyd SK, Bowman JL. 2004. Asymmetric leaf development and blade expansion in *Arabidopsis* are mediated by KANADI and YABBY activities. Development **131**, 2997–3006.

Feng X, Liu W, Dai H, Qiu Y, Zhang G, Chen ZH, Wu F. 2020. HvHOX9, a novel homeobox leucine zipper transcription factor revealed by root miRNA and RNA sequencing in Tibetan wild barley, positively regulates Al tolerance. Journal of Experimental Botany **71**, 6057–6073.

Floyd SK, Zalewski CS, Bowman JL. 2006. Evolution of class III homeodomain-leucine zipper genes in streptophytes. Genetics **173**, 373–388.

Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G. 2003. Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. Nature **426**, 147–153.

Gago GM, Almoguera C, Jordano J, Gonzalez DH, Chan RL. 2002. *Hahb-4*, a homeobox-leucine zipper gene potentially involved in abscisic acid-dependent responses to water stress in sunflower. Plant Cell and Environment **25**, 633–640.

Gehring WJ. 1987. Homeo boxes in the study of development. Science**236**, 1245–1252.

Gonzalez DH. 2016. Introduction to transcription factor structure and function. In: Gonzalez DH, ed. Plant transcription factors: evolutionary, structural and functional aspects. London: Academic Press, 3–11.

González FG, Capella M, Ribichich KF, Curín F, Giacomelli JI, Ayala F, Watson G, Otegui ME, Chan RL. 2019. Field-grown transgenic wheat expressing the sunflower gene *HaHB4* significantly outyields the wild type. Journal of Experimental Botany **70**, 1669–1681.

Grigg SP, Galinha C, Kornet N, Canales C, Scheres B, Tsiantis M. 2009. Repression of apical homeobox genes is required for embryonic root development in *Arabidopsis*. Current Biology **19**, 1485–1490.

Guan XY, Li QJ, Shan CM, Wang S, Mao YB, Wang LJ, Chen XY. 2008. The HD-Zip IV gene *GaHOX1* from cotton is a functional homologue of the *Arabidopsis GLABRA2*. Physiologia Plantarum **134**, 174–182.

Harris JC, Hrmova M, Lopato S, Langridge P. 2011. Modulation of plant growth by HD-Zip class I and II transcription factors in response to environmental stimuli. New Phytologist **190**, 823–837.

Hawker NP, Bowman JL. 2004. Roles for Class III HD-Zip and KANADI genes in Arabidopsis root development. Plant Physiology **135**, 2261–2270.

He G, Liu P, Zhao H, Sun J. 2020. The HD-ZIP II transcription factors regulate plant architecture through the auxin pathway. International Journal of Molecular Sciences **21**, 3250.

Henriksson E, Olsson AS, Johannesson H, Johansson H, Hanson J, Engström P, Söderman E. 2005. Homeodomain leucine zipper class I genes in Arabidopsis. Expression patterns and phylogenetic relationships. Plant Physiology **139**, 509–518.

Hong JC. 2016. General aspects of plant transcription factor families. In: Gonzalez DH, ed. Plant transcription factors: evolutionary, structural and functional aspects. London: Academic Press, 35–56.

Horstman A, Fukuoka H, Muino JM, Nitsch L, Guo C, Passarinho P, Sanchez-Perez G, Immink R, Angenent G, Boutilier K. 2015. AIL and HDG proteins act antagonistically to control cell proliferation. Development **142**, 454–464.

Ingram GC, Magnard JL, Vergne P, Dumas C, Rogowsky PM. 1999. *ZmOCL1*, an HDGL2 family homeobox gene, is expressed in the outer cell layer throughout maize development. Plant Molecular Biology **40**, 343–354.

Javelle M, Klein-Cosson C, Vernoud V, Boltz V, Maher C, Timmermans M, Depège-Fargeix N, Rogowsky PM. 2011. Genome-wide characterization of the HD-ZIP IV transcription factor family in maize: preferential expression in the epidermis. Plant Physiology **157**, 790–803.

Kilian J, Whitehead D, Horak J, Wanke D, Weinl S, Batistic O, D'Angelo C, Bornberg-Bauer E, Kudla J, Harter K. 2007. The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. The Plant Journal **50**, 347–363.

Köllmer I, Werner T, Schmülling T. 2011. Ectopic expression of different cytokinin-regulated transcription factor genes of *Arabidopsis thaliana* alters plant growth and development. Journal of Plant Physiology **168**, 1320–1327.

Kovalchuk N, Wu W, Bazanova N, *et al.* 2019. Wheat wounding-responsive HD-Zip IV transcription factor GL7 is predominantly expressed in grain and activates genes encoding defensins. Plant Molecular Biology **101**, 41–61.

Kubo H, Peeters AJ, Aarts MG, Pereira A, Koornneef M. 1999. *ANTHOCYANINLESS2*, a homeobox gene affecting anthocyanin distribution and root development in Arabidopsis. The Plant Cell **11**, 1217–1226.

Lavenus J, Goh T, Roberts I, Guyomarc'h S, Lucas M, De Smet I, Fukaki H, Beeckman T, Bennett M, Laplaze L. 2013. Lateral root development in *Arabidopsis*: fifty shades of auxin. Trends in Plant Science **18**, 450–458.

Lee HW, Kim NY, Lee DJ, Kim J. 2009. *LBD18/ASL20* regulates lateral root formation in combination with *LBD16/ASL18* downstream of *ARF7* and *ARF19* in Arabidopsis. Plant Physiology **151**, 1377–1389.

Li S, Chen N, Li F, Mei F, Wang Z, Cheng X, Kang Z, Mao H. 2020a. Characterization of wheat homeodomain-leucine zipper family genes and functional analysis of *TaHDZ5-6A* in drought tolerance in transgenic *Arabidopsis*. BMC Plant Biology **20**, 50.

Li T, Lei W, He R, Tang X, Han J, Zou L, Yin Y, Lin H, Zhang D. 2020b. Brassinosteroids regulate root meristem development by mediating BIN2-UPB1 module in *Arabidopsis*. PLoS Genetics **16**, e1008883.

Liu Y, Xu J, Guo S, *et al.* 2020. AtHB7/12 regulate root growth in response to aluminum stress. International Journal of Molecular Sciences **21**, 4080.

Liu Q, Yao X, Pi L, Wang H, Cui X, Huang H. 2009. The *ARGONAUTE10* gene modulates shoot apical meristem maintenance and establishment of leaf polarity by repressing miR165/166 in Arabidopsis. The Plant Journal **58**, 27–40.

Manavella PA, Dezar CA, Ariel FD, Chan RL. 2008. Two ABREs, two redundant root-specific and one W-box *cis*-acting elements are functional in the sunflower *HAHB4* promoter. Plant Physiology and Biochemistry **46**, 860–867.

Masucci JD, Rerie WG, Foreman DR, Zhang M, Galway ME, Marks MD, Schiefelbein JW. 1996. The homeobox gene *GLABRA2* is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana*. Development **122**, 1253–1260.

Meijer AH, de Kam RJ, d'Erfurth I, Shen W, Hoge JH. 2000. HD-Zip proteins of families I and II from rice: interactions and functional properties. Molecular & General Genetics **263**, 12–21.

Meijer AH, Scarpella E, van Dijk EL, Qin L, Taal AJ, Rueb S, Harrington SE, McCouch SR, Schilperoort RA, Hoge JH. 1997. Transcriptional repression by Oshox1, a novel homeodomain leucine zipper protein from rice. The Plant Journal **11**, 263–276.

Miao ZQ, Zhao PX, Mao JL, Yu LH, Yuan Y, Tang H, Liu ZB, Xiang CB. 2018. HOMEOBOX PROTEIN52 mediates the crosstalk between ethylene and auxin signaling during primary root elongation by modulating auxin transport-related gene expression. The Plant Cell **30**, 2761–2778.

Mukherjee K, Brocchieri L, Bürglin TR. 2009. A comprehensive classification and evolutionary analysis of plant homeobox genes. Molecular Biology and Evolution **26**, 2775–2794.

Mukherjee K, Burglin TR. 2006. MEKHLA, a novel domain with similarity to PAS domains, is fused to plant homeodomain-leucine zipper III proteins. Plant Physiology **140**, 1142–1150.

Müller CJ, Valdés AE, Wang G, Ramachandran P, Beste L, Uddenberg D, Carlsbecker A. 2016. PHABULOSA mediates an auxin signaling loop to regulate vascular patterning in Arabidopsis. Plant Physiology **170**, 956–970.

Nakamura M, Katsumata H, Abe M, Yabe N, Komeda Y, Yamamoto KT, Takahashi T. 2006. Characterization of the class IV homeodomain-leucine zipper gene family in Arabidopsis. Plant Physiology **141**, 1363–1375.

Ohashi Y, Oka A, Rodrigues-Pousada R, Possenti M, Ruberti I, Morelli G, Aoyama T. 2003. Modulation of phospholipid signaling by GLABRA2 in root-hair pattern formation. Science **300**, 1427–1430.

Osmont KS, Sibout R, Hardtke CS. 2007. Hidden branches: developments in root system architecture. Annual Review of Plant Biology **58**, 93–113.

Perotti MF, Arce AL, Chan RL. 2021. Data from: The underground life of homeodomain-leucine zipper transcription factors. Dryad Digital Repository. https://doi.org/10.5061/dryad.mpg4f4qzb

Perotti MF, Ariel FD, Chan RL. 2020. Lateral root development differs between main and secondary roots and depends on the ecotype. Plant Signaling & Behavior **15**, 1755504.

Perotti MF, Ribone PA, Cabello JV, Ariel FD, Chan RL. 2019. AtHB23 participates in the gene regulatory network controlling root branching, and reveals differences between secondary and tertiary roots. The Plant Journal **100**, 1224–1236.

Perotti MF, Ribone PA, Chan RL. 2017. Plant transcription factors from the homeodomain-leucine zipper family I. Role in development and stress responses. IUBMB Life **69**, 280–289.

Petricka JJ, Winter CM, Benfey PN. 2012. Control of *Arabidopsis* root development. Annual Review of Plant Biology **63**, 563–590.

Ré DA, Capella M, Bonaventure G, Chan RL. 2014. Arabidopsis *AtHB7* and *AtHB12* evolved divergently to fine tune processes associated with growth and responses to water stress. BMC Plant Biology **14**, 150.

Renau-Morata B, Carrillo L, Dominguez-Figueroa J, Vicente-Carbajosa J, Molina RV, Nebauer SG, Medina J. 2020. CDF transcription factors: plant regulators to deal with extreme environmental conditions. Journal of Experimental Botany **71**, 3803–3815.

Ribichich KF, Chiozza M, Ávalos-Britez S, *et al.* 2020. Successful field performance in warm and dry environments of soybean expressing the sunflower transcription factor HB4. Journal of Experimental Botany **71**, 3142–3156.

Rogg LE, Lasswell J, Bartel B. 2001. A gain-of-function mutation in *IAA28* suppresses lateral root development. The Plant Cell **13**, 465–480.

Ruan L, Chen L, Chen Y, He J, Zhang W, Gao Z, Zhang Y. 2012. Expression of *Arabidopsis HOMEODOMAIN GLABROUS 11* enhances tolerance to drought stress in transgenic sweet potato plants. Journal of Plant Biology **55**, 151–158.

Ruberti I, Sessa G, Lucchetti S, Morelli G. 1991. A novel class of plant proteins containing a homeodomain with a closely linked leucine zipper motif. The EMBO Journal **10**, 1787–1791.

Salvi E, Rutten JP, Di Mambro R, Polverari L, Licursi V, Negri R, Dello Ioio R, Sabatini S, Ten Tusscher K. 2020. A self-organized PLT/Auxin/ARR-B network controls the dynamics of root zonation development in *Arabidopsis thaliana*. Developmental Cell **53**, 431–443. e23.

Sawa S, Ohgishi M, Goda H, Higuchi K, Shimada Y, Yoshida S, Koshiba T. 2002. The *HAT2* gene, a member of the HD-Zip gene family, isolated as an auxin inducible gene by DNA microarray screening, affects auxin response in *Arabidopsis*. The Plant Journal **32**, 1011–1022.

Schena M, Davis RW. 1992. HD-Zip proteins: members of an *Arabidopsis* homeodomain protein superfamily. Proceedings of the National Academy of Sciences, USA **89**, 3894–3898.

Schrick K, Nguyen D, Karlowski WM, Mayer KF. 2004. START lipid/sterol-binding domains are amplified in plants and are predominantly associated with homeodomain transcription factors. Genome Biology **5**, R41.

Sessa G, Carabelli M, Possenti M, Morelli G, Ruberti I. 2018. Multiple links between HD-Zip proteins and hormone networks. International Journal of Molecular Sciences **19**, 4047.

Silva AT, Ribone PA, Chan RL, Ligterink W, Hilhorst HW. 2016. A predictive coexpression network identifies novel genes controlling the seed-to-seedling phase transition in *Arabidopsis thaliana*. Plant Physiology **170**, 2218–2231.

Singh A, Roy S, Singh S, Das SS, Gautam V, Yadav S, Kumar A, Singh A, Samantha S, Sarkar AK. 2017. Phytohormonal crosstalk modulates the expression of miR166/165s, target *Class III HD-ZIPs*, and *KANADI* genes during root growth in *Arabidopsis thaliana*. Scientific Reports **7**, 3408.

Singh A, Singh S, Panigrahi KC, Reski R, Sarkar AK. 2014. Balanced activity of *microRNA166/165* and its target transcripts from the class III homeodomain-leucine zipper family regulates root growth in *Arabidopsis thaliana*. Plant Cell Reports **33**, 945–953.

Smetana O, Mäkilä R, Lyu M, *et al.* 2019. High levels of auxin signalling define the stem-cell organizer of the vascular cambium. Nature **565**, 485–489.

Söderman E, Hjellström M, Fahleson J, Engström P. 1999. The HD-Zip gene *ATHB6* in *Arabidopsis* is expressed in developing leaves, roots and carpels and up-regulated by water deficit conditions. Plant Molecular Biology **40**, 1073–1083.

Son O, Cho HY, Kim MR, *et al.* 2005. Induction of a homeodomain–leucine zipper gene by auxin is inhibited by cytokinin in *Arabidopsis* roots. Biochemical and Biophysical Research Communications **326**, 203–209.

Steindler C, Matteucci A, Sessa G, Weimar T, Ohgishi M, Aoyama T, Morelli G, Ruberti I. 1999. Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. Development **126**, 4235–4245.

Taiz L, Zeiger E. 2006. Plant Physiology, 4th ed. Sunderland: Sinauer Associates.

Tron AE, Bertoncini CW, Chan RL, Gonzalez DH. 2002. Redox regulation of plant homeodomain transcription factors. Journal of Biological Chemistry **277**, 34800–34807.

Turchi L, Baima S, Morelli G, Ruberti I. 2015. Interplay of HD-Zip II and III transcription factors in auxin-regulated plant development. Journal of Experimental Botany **66**, 5043–5053.

Turchi L, Carabelli M, Ruzza V, *et al.* 2013. *Arabidopsis* HD-Zip II transcription factors control apical embryo development and meristem function. Development **140**, 2118–2129.

Valle EM, Gonzalez DH, Gago G, Chan RL. 1997. Isolation and expression pattern of *hahr1*, a homeobox-containing cDNA from *Helianthus annuus*. Gene **196**, 61–68.

Vollbrecht E, Veit B, Sinha N, Hake S. 1991. The developmental gene *Knotted-1* is a member of a maize homeobox gene family. Nature **350**, 241–243.

Wang X, Bi S, Wang L, *et al.* 2020. GLABRA2 regulates actin bundling protein VILLIN1 in root hair growth in response to osmotic stress. Plant Physiology **184**, 176–193.

Wei M, Liu Q, Wang Z, *et al.* 2020. PuHox52-mediated hierarchical multilayered gene regulatory network promotes adventitious root formation in *Populus ussuriensis*. New Phytologist **228**, 1369–1385.

Wendrich JR, Yang BJ, Vandamme N, *et al.* 2020. Vascular transcription factors guide plant epidermal responses to limiting phosphate conditions. Science **370**, eaay4970.

Wenkel S, Emery J, Hou BH, Evans MM, Barton MK. 2007. A feedback regulatory module formed by LITTLE ZIPPER and HD-ZIPIII genes. The Plant Cell **19**, 3379–3390.

Xu P, Cai XT, Wang Y, Xing L, Chen Q, Xiang CB. 2014. HDG11 upregulates cell-wall-loosening protein genes to promote root elongation in *Arabidopsis*. Journal of Experimental Botany **65**, 4285–4295.

Xu P, Zhao PX, Cai XT, Mao JL, Miao ZQ, Xiang CB. 2020. Integration of jasmonic acid and ethylene into auxin signaling in root development. Frontiers in Plant Science **11**, 271.

Yu H, Chen X, Hong YY, Wang Y, Xu P, Ke SD, Liu HY, Zhu JK, Oliver DJ, Xiang CB. 2008. Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. The Plant Cell **20**, 1134–1151.

Yu L, Chen X, Wang Z, Wang S, Wang Y, Zhu Q, Li S, Xiang C. 2013. Arabidopsis *Enhanced Drought Tolerance1/HOMEODOMAIN GLABROUS11* confers drought tolerance in transgenic rice without yield penalty. Plant Physiology **162**, 1378–1391.

Zhang H, Ma X, Li W, Niu D, Wang Z, Yan X, Yang X, Yang Y, Cui H. 2019. Genome-wide characterization of NtHD-ZIP IV: different roles in abiotic stress response and glandular trichome induction. BMC Plant Biology **19**, 444.

Zhang TQ, Xu ZG, Shang GD, Wang JW. 2019. A single-cell RNA sequencing profiles the developmental landscape of *Arabidopsis* root. Molecular Plant **12**, 648–660.

Zhao Y, Zhou Y, Jiang H, Li X, Gan D, Peng X, Zhu S, Cheng B. 2011. Systematic analysis of sequences and expression patterns of drought-responsive members of the HD-Zip gene family in maize. PLoS One **6**, e28488.

Zheng G, Fan C, Di S, Wang X, Xiang C, Pang Y. 2017. Over-expression of *Arabidopsis* EDT1 gene confers drought tolerance in alfalfa (*Medicago sativa* L.). Frontiers in Plant Science **8**, 2125.