PROCEEDINGS B

rspb.royalsocietypublishing.org

Review

Cite this article: Di Ruocco G, Di Mambro R, Dello Ioio R. 2018 Building the differences: a case for the ground tissue patterning in plants. *Proc. R. Soc. B* **285**: 20181746.
http://dx.doi.org/10.1098/rspb.2018.1746

Received: 2 August 2018
Accepted: 12 October 2018

Subject Category:
Development and physiology

Subject Areas:
plant science, developmental biology, evolution

Keywords:
ground tissue, plants, root development, asymmetric cell division, *Arabidopsis thaliana*, cortex

Authors for correspondence:
Riccardo Di Mambro
e-mail: riccardo.dimambro@unipi.it
Raffaele Dello Ioio
e-mail: raffaele.delloioio@uniroma1.it

THE ROYAL SOCIETY PUBLISHING

Building the differences: a case for the ground tissue patterning in plants

Giovanna Di Ruocco[1], Riccardo Di Mambro[2] and Raffaele Dello Ioio[1]

[1]Laboratory of Functional Genomics and Proteomics of Model Systems, Dipartimento di Biologia e Biotecnologie, Sapienza Università di Roma, Via dei Sardi 70, 00185 Rome, Italy
[2]Dipartimento di Biologia, Università di Pisa, via Luca Ghini, 13-56126 Pisa, Italy

RDM, 0000-0002-1243-7395; RDI, 0000-0001-8679-9412

A key question in biology is to understand how interspecies morphological diversities originate. Plant roots present a huge interspecific phenotypical variability, mostly because roots largely contribute to adaptation to different kinds of soils. One example is the interspecific cortex layer number variability, spanning from one to several. Here, we review the latest advances in the understanding of the mechanisms expanding and/or restricting cortical layer number in *Arabidopsis thaliana* and their involvement in cortex pattern variability among multi-cortical layered species such as *Cardamine hirsuta* or *Oryza sativa*.

1. Introduction

How different morphologies originate in nature is a fundamental question in biology. Researchers in evolutionary developmental biology are trying to shed light on the molecular mechanisms underlying the differences in shape and anatomy that allow organisms to cope with the large diversity of environments on land. In this view, one challenge is the identification of feasible model systems to study differences in development. A breakthrough approach to isolate the genetic mechanisms at the basis of phenotypical differences is the use of comparative studies [1–3]. Most of the success of this strategy consists in the identification of genetic differences underlying phenotypical diversity over a short evolutionary scale, such as differences in gene activity and/or expression.

Plant roots represent an ideal model system for comparative anatomy studies: (i) plant root anatomy largely varies among species; and (ii) roots are transparent and have simple anatomy, allowing fine and precise microscopy analysis of differences in anatomical traits between plant species. The roots of most plants have a radial symmetry and can be represented by a series of concentric cylinders. Briefly, the outer cylinder represents the epidermis, and the inner cylinders represent the cortex layer(s), the endodermis, the pericycle and the vascular bundle [4,5]. Cortex(es) and the endodermis form all together the ground tissue (GT) (figure 1) [4,5]. All root tissues originate from a set of initials/stem cells located in specialized region at the tip of the root, called root apical meristem (RAM) [6–8].

The extraordinary diffusion of plants in multiple different environments is partly due to the fundamental role of roots in anchoring plants to soil and allowing the uptake of water and nutrients. Root cortical tissue plays a fundamental role in permitting plants to cope with a variety of environments. In plants living in wet soils, such as rice, the control of water/air ratio is determined by the formation of the aerenchyma, a specialized tissue derived from root cortex secondary growth [9]. In plants such as turnip or horseradish living in adverse weather conditions, root cortex originates storage parenchyma, a tissue where simple carbohydrates are converted in starch [9]. In legumes such as *Medicago sativa* and *truncatula*, the dedifferentiation processes of cortex cells give rise to the symbiotic nodule, where the symbiosis with nitrogen-fixing rhyzobia takes place [10,11]. Moreover, it was recently shown that plants such as *Arabidopsis thaliana* and *Hakea actites* uptake proteins from soil

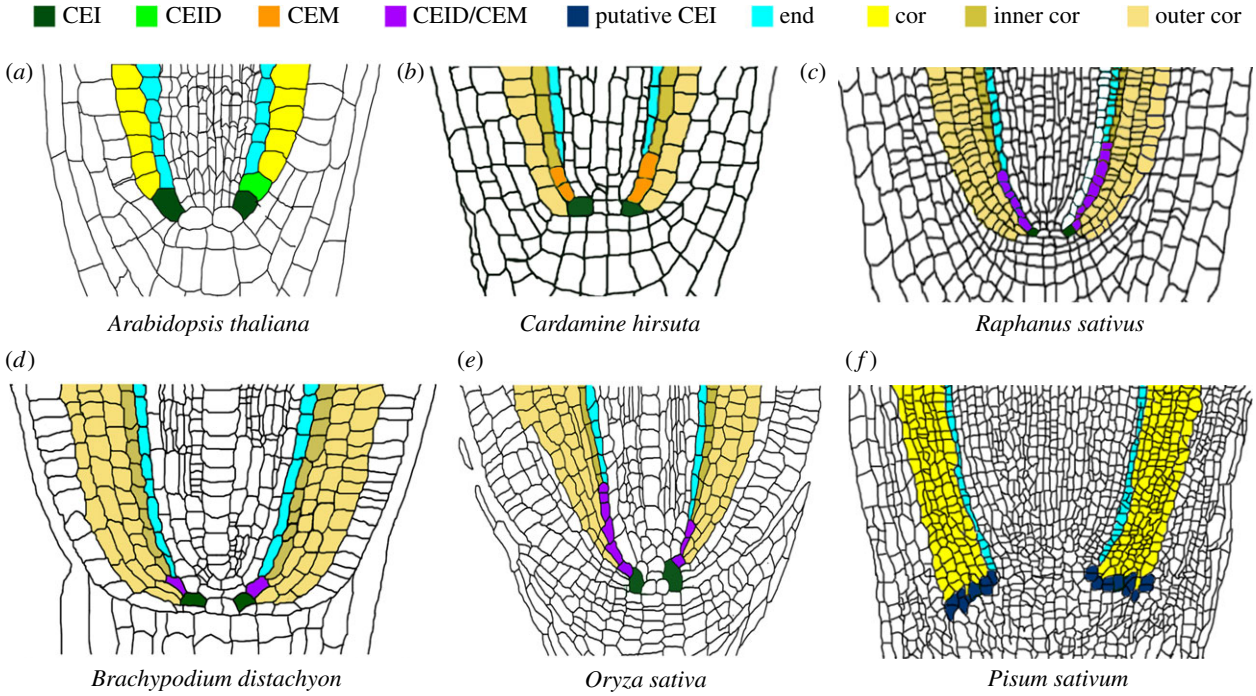

Figure 1. GT cell fate in different species. Cartoon representing longitudinal sections of (*a*) *Arabidopsis thaliana*, (*b*) *Cardamine hirsuta*, (*c*) *Raphanus sativum*, (*d*) *Brachypodium distachyon*, (*e*) *Oryza sativa* and (*f*) *Pisum sativum* root meristem. Abbreviations in colour key at top of figure: end, endodermis; cor, cortex; CEM, mixed cortex and endodermis identity cell. Note that the number of cortex layers varies among species.

and transfer them into cortex as nitrogen source [12]. Hence, modulating the number of cortical layers, plants can model their resistance to stresses and changes in the environment; therefore, the understanding of the molecular mechanism controlling cortex layer number is of primary interest in plant biology.

Cortex origin and architecture largely vary depending on the phyletic origin (monocots versus dicots) and on the type of RAM: open RAM is characterized by the absence of boundaries between specific tissues in the growing tip and closed RAM has distinct boundaries between apical regions that can be identified [13,14]. Some species of plants presenting a closed RAM (e.g. the model system *A. thaliana*) have one cortex layer at seedling stage, whereas some other species show multiple cortical layers (e.g. *Cardamine hirsuta*, *Raphanus sativus*, *Hordeum vulgare*, *Brachypodium distachyon* or *Oryza sativa*; figure 1) [15,16]. Furthermore, cortical layer number can also vary in the same organism. *Arabidopsis* acquires one cortex layer more, called middle cortex (MC), during development [17,18], whereas *O. sativa* shows a complicated cortex layer number pattern: rice embryo primary roots (radicule) show five cortex layers, whereas the lateral and crown roots show a variable number between 1 and 10 [15,19,20]. In species presenting an open meristem such as *Pisum sativum* (pea), there are no specific tiers of cortical initials [21]. Morphological analysis suggests that cortical initials in pea are distributed in continuous layers located over the columella [13] (figure 1).

Such interspecific variability of cortex layer number epitomizes an extraordinary resource for comparative anatomy studies. The tremendous technological advance of recent years made more approachable research on a huge variety of model systems, enhancing the knowledge on root patterning. In this review, we aim to shed light on the differences in molecular pathways subtending GT variability among species based on (i) the most recent advances in understanding the GT patterning in *Arabidopsis* and (ii) the latest findings in understanding the mechanisms controlling cortical layer number in *Arabidopsis* close and distant relatives.

2. Root cortex patterning in *Arabidopsis*

The GT (cortex and the endodermis) originates from the RAM, a region located at the tip of the root where a set of self-renewing stem cells divide producing all the root tissues. In *Arabidopsis*, a stem cell, called cortex endodermis initial (CEI), gives rise to the GT (figure 2). An asymmetric anticlinal division of CEI gives rise to a self-renewed stem cell (CEI) and to a daughter cell (CEID) (figure 2). Subsequently, a periclinal division occurs in the CEID generating endodermis and cortex [4] (figure 2). The CEID periclinal division occurs already in late embryo development, determining the formation of an embryonic cortex and endodermis and, therefore, defining the tissue organization of the primary root [4]. During post-embryonic development, an additional periclinal division occurs in the meristematic endodermis giving rise to a novel cortex layer, the MC [17] (figure 2).

(a) A genetic network controls endodermis and cortex patterning

In recent years, several genetic pathways controlling the CEID periclinal division have been discovered in *Arabidopsis*. GRAS family transcription factors SHORT-ROOT (SHR) and SCARECROW (SCR) are the main fate determinant of the GT, as suggested by monolayered GT in *shr* and *scr* mutants [22,23]. Consistent with their fundamental role in GT development, *SHR* is expressed in the vascular tissue, but moves to the CEI, CEID and endodermis via plasmodesmata (PD)

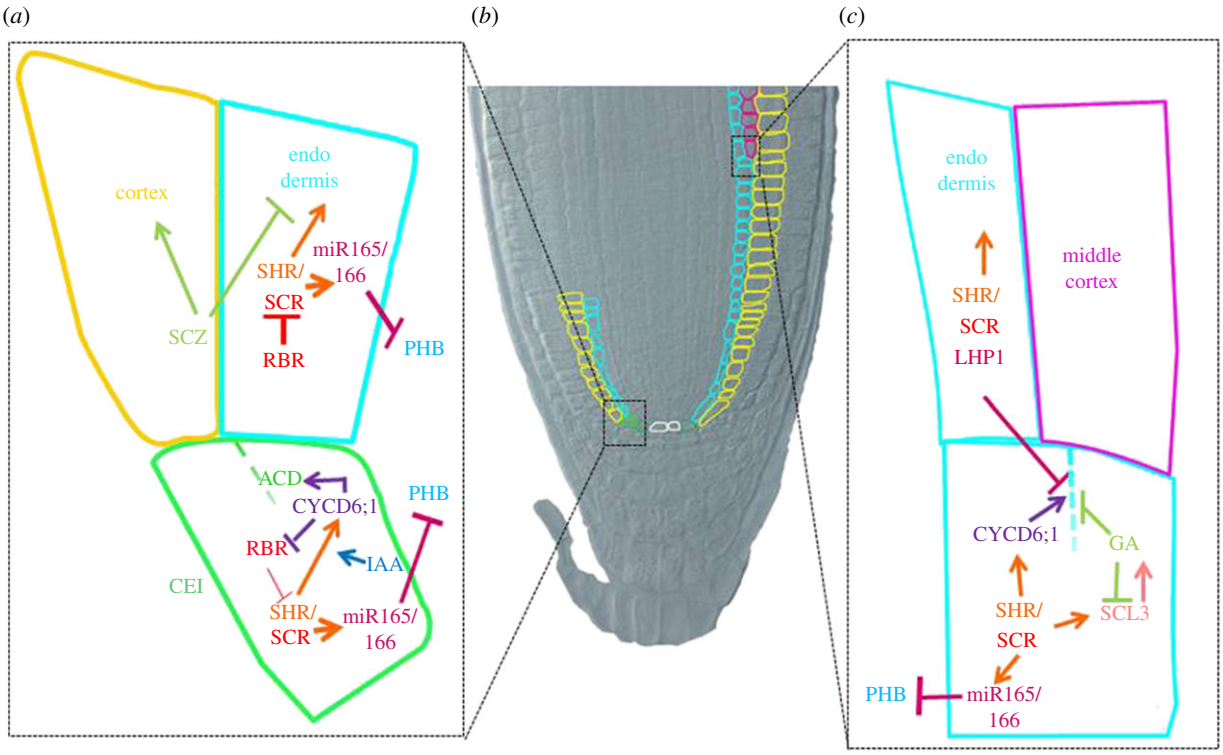

Figure 2. Model depicting the main pathways contributing to asymmetric cell division in *Arabidopsis* root. (*a*) Cartoon representing *Arabidopsis* CEI and endodermis and cortex. In *Arabidopsis* CEI (in green), a SHR/SCR complex promotes ACD via activation of CYCD6;1 (CYCD6;1). High levels of Auxin (IAA) in the CEI contribute to CYCD6 activation, whereas CYCD6 inactivates RBR, promoting SCR activity. In the endodermis cells (light blue), RBR binds to SCR inhibiting cell division, whereas SHR instructs endodermis fate. In the cortex (yellow), SCZ promotes cortex fate and inhibits endodermis identity. SHR/SCR complex promotes miR165/6 expression restricting PHB to the stele. (*b*) Nomarski differential contrast interference image depicting 10 days after germination of *Arabidopsis* root meristem. GT cells are coloured: green (CEI), light blue (endodermis), yellow (cortex), white (QC), purple (MC). (*c*) Cartoon representing *Arabidopsis* endodermis and MC. In *Arabidopsis* endodermis (light blue), SCR/LHP1 complex inhibits division. When MC (purple) is forming, SCR in complex with SHR induces CYCD6 and, hence, ACD. SCL3, a target of SCR and GA, inhibits ACD and sustains GA activity. GA inhibits ACD (dashed line).

[24–27], whereas *SCR* is expressed in the CEI, CEID and endodermis, and sequesters SHR to the nucleus in those cells [22,24].

In the CEID, SHR directly activates *SCR*, increasing its expression and regulating the periclinal division of the CEID [28–31] (figure 2). Once that SCR sequesters SHR to the nucleus, they form an active transcriptional complex and together they regulate CEID division promoting the expression of *CYCLIND6;1* (*CYCD6;1*) [29,30]. The SHR/SCR complex induces the expression of the BIRD Zinc finger proteins NUTCRACKER (NUT), JACKDAW (JKD), MAGPIE (MGP) and BALDIBIS (BIB) that act in concert with SCR to reduce SHR movements, thus establishing and maintaining the boundaries between stele and GT [26,30,32].

SHR/SCR module controls also the CEI to CEID transition acting via a bi-stable circuit that integrates radial and longitudinal information and regulates cell cycle progression [28,32–34].

Indeed, SCR directly interacts with the orthologue of the animal cell cycle regulator RETINOBLASTOMA-RELATED (RBR) protein. The RBR/SCR/SHR interaction reduces SHR/SCR complex formation and hence inhibits CEI division [32]. The molecular mechanism guiding the periclinal division of the CEID relies on the negative activity of the CYCD6;1 on RBR: CYCD6;1 mediates RBR phosphorylation, and hence its inactivation. CEID asymmetric cell division (ACD) and GT fate specification depend also on hormone activity. The longitudinal gradient of auxin, a plant hormone with morphogen-like characteristics [35], positively influences *CYCD6;1* transcription supporting SHR/SCR complex formation and RBR phosphorylation. Subsequently, the decreased auxin levels reduce *CYCD6;1* expression, allowing RBR to negatively regulate cell division and sustaining the acquirement of cortical and endodermal cell fate.

Interestingly, whereas SHR is required for determining endodermis specification, SCR is not involved in this process; indeed, *scr* monolayered GT shows both cortical and endodermal identity, whereas the *shr* one shows only cortex identity. Recently, it has been proposed that SHR promotes the expression of genes involved in Casparian strip formation independently of SCR, confirming the central role of SHR in determining endodermis identity [36].

Recent findings have also involved auxin in GT initiation during embryogenesis, as the auxin-dependent transcription factor MONOPTEROS (MP/AUXIN RESPONSIVE FACTOR 5/ARF5) promotes the establishment of the GT acting directly on the progenitor cells of the GT [37]. Intriguingly, MP does not require SHR/SCR module for initiating the GT, suggesting that these genes are involved mostly in the regulation of the formative divisions of a pre-formed GT layer rather than in the formation of this tissue. Further studies will be required to elucidate the molecular factors through which MP regulates GT initiation. Also, Moller *et al.* [37] suggest that MP activity is necessary for promoting *SHR* and *SCR* expression, as those genes are strongly downregulated in *mp* mutants, thus involving auxin also in the maintenance of GT patterning. Nonetheless, lack of the GT itself in *mp* mutants makes it difficult to understand whether

MP genuinely maintains GT patterning or only regulates GT establishment.

(b) Restriction mechanism of ground tissue proliferation

The genetic mechanisms described above are necessary and sufficient to initiate and maintain formative divisions. Nevertheless, other independent mechanisms determine the confinement of the GT and fate division. One of the best-known transcription factors involved in GT fate separation is *SCHIZORIZA* (*SCZ*). SCZ is a transcription factor member of the Heat Shock family genes necessary for root cell fate separation and cortex identity (figure 2), as *scz* loss-of-function mutants show extra layers in the root expressing both epidermal, endodermal and cortical markers, whereas *SCZ* overexpressors show ectopic cortex identity specification [38,39]. Expression of *SCZ* in the cortex is sufficient to rescue the cortex extra layer formation in *scz* mutant background, suggesting that SCZ is required for cortex cell fate specification [39]. Future studies will help to clarify how *SCZ* controls fate separation and define cortex identity.

microRNAs (*miRs*), small RNA fragments acting as a repressor, have largely been linked to the GT patterning [40–42]. Mutants in genes involved in *miRs* biogenesis and function, such as *HYPONASTIC LEAVES* (*HYL1*) and *ARGONAUTE1* (*AGO1*) involved in miRNA cleavage and target recognition, respectively, exhibit additional layers in the GT [41,43], suggesting an active role for *miRs* in GT boundary definition. In *Arabidopsis microRNA165/6* family, members regulate the spatial distribution of HD-ZIPIII (HOMEODOMAIN LEUCINE ZIPPER III) family transcription factors such as *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*). miR165/6/PHB/PHV module is involved in GT development, as transgenics with reduced miR165/6 activity and miR165/6 insensitive PHB and PHV mutants show additional cortical layers. It was shown that SHR regulates the expression of the four *miR165/6* loci expressed in the GT (figure 2) [40]. Once produced in this tissue, miR165/6 migrates towards the stele via PD generating a radial gradient with a maximum in the GT and a minimum in the stele [40]. This movement results in a miR165/6 dose-dependent restriction of PHB expression that specify both xylem differentiation and GT patterning (figure 2) [40,41]. The SHR-dependent *miR165/6* expression does not rule out the possibility of a parallel activity of miR165/6 to SHR and SCR in GT specification as residual expression of *miR165/6* is detectable in *shr* mutant root [40–42]. Identification of additional components regulating miR165/6 in the GT will permit to better clarify the involvement of these genes in GT development. Moreover, it has been shown that PHB modulates *CYCD6;1* expression independently on SHR [43]. Nevertheless, how the two pathways interact in the GT patterning is still an object of study.

Interestingly, small signalling molecules, such as peptides, also contribute to boundaries formation via robust transcription factor activity confinement [44]. In root, CLV3/EMBRYO SURROUNDING REGION-RELATED (CLE) peptides control root meristem size as their ectopic applications cause root meristem size reductions and additional cortical layer production [45]. PUB4, a plant U-box E3 ubiquitin ligase, was isolated as a downstream factor of CLV peptides in controlling ACDs timing [46]. Hence, cortical layer number restriction is based on the establishment and maintenance of positional information whose variation leads to a GT patterning alteration. How all those mechanisms interact is still a matter of discussion.

(c) Mechanisms controlling middle cortex development

In *Arabidopsis*, an additional cortex layer, called MC for its anatomical position, is formed between 7 and 10 days after germination [17,18,47]. Instead of originating from a periclinal division occurring in the CEID, the MC originates from a periclinal asynchronous division of an endodermis cell far from the QC. This division gives rise to a new layer with cortex identity [17] (figure 2).

SHR and *SCR* play a major role in MC formation, despite acting antagonistically in this context [15,18,48]. SHR-dependent reactivation of *CYCD6;1* in the endodermis is necessary and sufficient to drive a formative endodermal division that will give rise to the MC (figure 2) [29]. On the contrary, SCR represses MC development (figure 2), as shown by in *scr* hypomorphic mutants [18].

Interestingly, SCR presents a dichotomous behaviour depending on its different interactors in the CEI and MC. In MC development, the interaction between SCR and the chromodomain-containing protein LIKE HETEROCHROMATIN PROTEIN1 (LHP1) determines the repression of the ACD in the GT responsible for MC formation. In accordance, *lhp1* mutants show premature formation of the second longitudinal ACD similarly to *scr* mutants and several SHR/SCR targets are repressed by this gene. Hence, SCR might induce or repress formative divisions depending on the amount of SCR interacting with SHR or with LHP1, respectively [49]. Interestingly, LHP1 might act both as a positive and negative regulator, as in the shoot it promotes the expression of the auxin synthesis gene YUCCA4 [50]. Recent findings have shown that other epigenetic factors are also involved in GT development, for example the histone deacetylase (HDAC) family HDA19 interacts with SCR in the CEI and thus affects cortical cell fate [51]. LHP1 and HDA19 involvement in GT patterning highlights the major contribution of epigenetic regulation in post-embryonic development. In future, it will be interesting to understand how epigenetic control interferes with patterning, and whether it is related to plant ageing and/or growth environment.

Among the specific mechanisms for MC development, the plant hormone gibberellin (GA) was found to have a significant role in MC formation timing. Indeed, GA treatments are sufficient to delay the formative division of the endodermis from which the MC originates, whereas plants treated with the GA inhibitor Paclobutrazol show premature formation of MC [18,49]. SCR/SHR and GA pathways convey on the regulation of the transcriptional regulator *SCARECROW LIKE 3* (*SCL3*) in MC development. Indeed, *SCL3* is activated by SHR/SCR and repressed by GA [28,52] (figure 2). Interestingly, *SCL3* regulates positively GA activity, controlling the timing of MC formation (figure 2).

Recently, a vacuolar sorting protein involved in protein recycling and interacting with SHR, *SHRUBBY* (*SHBY*), was shown to play a role in integrating SCR/SHR and GA pathways [53]. SHBY inhibits SHR activity in the MC and positively regulates GA signalling via an unidentified mechanism, preventing the formative division generating the MC [53]. This interesting finding suggests that MC formation is not only subject to a tight transcriptional regulation, but it is also finely regulated by protein turnover.

An interesting case in MC patterning is represented by the role of *SPINDLY* (*SPY*). SPY is an O-linked glucosamine acetyltransferase with GA response–repressive functions [54]. Although *spy* mutants present high GA levels, premature MC formation can be observed in this background [52]. Because SPY homologues in animals interact with histone deacetylase [55], recent theories posit that SPY might control MC formation epigenetically. Nevertheless, the lack of direct evidence of SPY involvement in epigenetic MC control does not exclude the possibility of additional molecular pathways controlled by SPY. For instance, it was recently shown that SPY has a role in maintaining cellular redox homeostasis and that oxidative stress induces MC formation [56]. In particular, premature MC formation in the *spy* mutant is suppressed by a reducing agent, while it is induced by H_2O_2 treatment [54]. This suggests that the increase in the number of cortex layers is a developmental response to oxidative stress. In this way, this regulation of cortex proliferation would result in a protective response carried out by plants to limit the entry of harmful elements and maintain a healthy redox state of the cell. In accordance, *spy* mutant is more tolerant to high salt concentration in the soil [57], supporting the idea that the cortex contributes to counteracting soil-based abiotic stress [58].

(d) Mechanisms of cortex proliferation in species with multiple cortex layer

In nature cortex, proliferation represents a chance for plants to adapt to their ecological niches. In rice roots, overexpression of the NAC domain protein *NAC10* results in enhanced root diameter due to increased cortical, epidermal and stele size. Intriguingly, those plants are better adapted to stress than the wild-type ones, most probably thanks to their cortical system [55]. Most of the species, from ferns to angiosperms, present several cortical layers. The additional formative divisions, at the basis of those multiple cortex layer formation, happen in precise positions early in development [13]. As the model system *Arabidopsis* develops only one cortical layer, the isolation of a feasible model system for multiple cortex layer development is mandatory. In *O. sativa*, the most diffused monocot model system, root architecture, is compounded by a series of adventitious roots, called crown roots, surrounding a primary root (radicule), carrying several lateral roots [56]. All the roots of rice present similar anatomy with the exception of different cortex layer number (one in the lateral roots and over 10 in the crown roots) [15,20,59]. In the rice stem cell niche, the CEI gives rise to the epidermis and the CEID. An additional formative division of the CEID originates endodermis and cortex [15]. Subsequently, other periclinal asymmetric divisions occur, giving rise to multiple cortex layers [15]. Usage of immunohistochemical markers suggests a different identity of inner and outer rice cortical layers [20]. Future studies will clarify the physiological and developmental differences among the two tissues.

In recent years, several findings have suggested an active role for SHR in cortical layer number determination in rice. Two orthologues of *SHR* and *SCR* are present in the rice genome. *In situ* hybridization and two hybrid system experiments on these genes support the idea that the OsSHR/SCR module controls endodermis development similarly to *Arabidopsis*, where SHR movements are limited to the endodermis by the interaction with SCR determining the identity of this tissue [28,60]. Nevertheless, it is still debated whether SHR plays a central role in cortical layer number. Corroborating this hypothesis, OsSHR2 or a *B. distachyon* orthologue of *SHR* (BdSHR) in *Arabidopsis* is sufficient to generate extra layers with cortex identity in *Arabidopsis* [61]. In *Arabidopsis*, OsSHR1/OsSHR2/BdSHR moves from the stele to the cortex, triggering the SCR/CYCD6;1 circuit and, hence, causing extra divisions in the GT [61]. Moreover, transgenic rice plants overexpressing SHR show an increase in outer cortical layer number [62]. Immunolocalizations of SHR2 in rice have shown that SHR2 protein is detectable in both endodermis and outer cortical layers. These data suggest that SHR might play a key role in multi-layered cortical patterning; nevertheless, the lack of OsSHR1/2 and OsSCR1/2 mutants and tissue-specific complementation makes it difficult to understand the specific role of SHR/SCR in rice GT development.

(e) *Cardamine hirsuta*, a model system for comparative development studies: a future perspective

The usage of closely related species has emerged as a successful strategy to understand the molecular differences that underline interspecific variability [1,63–65]. Among the close relative of *Arabidopsis* exhibiting multiple cortical layers, *C. hirsuta* represents a breakthrough in our understanding of the genetic basis of root anatomical diversity. In recent years, *Cardamine* emerged as a powerful system to identify molecular mechanisms at the base of biological diversity in leaf morphology and petal and fruit development [66–73]. *Cardamine* suits most of the characteristic of a model system. It is diploid, it possesses a completely sequenced and annotated small genome (196 mega bases) [74,75], it is self-compatible and it has a short life cycle (about four months) [74]. *Cardamine* can be transformed via *Agrobacterium tumefaciens*-based floral dip methodology with a high efficiency [74]. The readiness of the genetic tractability of *Cardamine* permits the exploitation of genetic screens and gene expression analysis and manipulation. *Cardamine* and *Arabidopsis* present several morphological divergent traits in the root [74]. Macroscopically, *Cardamine* primary root emerges from seed and produces several adventitious roots about 6 days after germination. Microscopically, *Cardamine* primary root presents anatomical traits divergent from *Arabidopsis* such as root meristem size, statoliths distribution and number of cortical layers [74]. Accurate analysis of *Cardamine* root GT patterning demonstrated that *Cardamine* show two cortical layers (an outer and an inner one) originating from a developmental domain of mix cortex and endodermis identity (CEM) absent in *Arabidopsis*. In this species, the CEI firstly divides asymmetrically giving rise to a cortex layer and a CEM that subsequently divides periclinally originating the endodermis and an inner cortex (figure 2*b*) [43]. The *Cardamine* inner and outer cortical layers are patterned by stereotypical division happening during embryogenesis. We recently found out that HD-ZIPIII members pattern *Cardamine* GT. In *Cardamine*, as in *Arabidopsis*, five loci encoding five HD-ZIPIII transcription factors (ChPHABULOSA; ChPHAVALUTA; ChCORONA; ChREVOLUTA; ChHB8) are present. As in *Arabidopsis*, their expression is modulated by the activity of miR165/6. In *Cardamine*, knockdown of those transcription factors results in the absence of the inner cortical layer, suggesting that the activity of those genes is necessary for

Cardamine additional cortex formation [43]. Intriguingly, in *Cardamine*, miR165/6 activity is low in the CEM, generating a broader expression domain of PHB that is therefore expressed in this tissue [43]. As in *Arabidopsis* PHB directs *CYCD6;1* expression, it is tempting to speculate that PHB directs formative divisions enriching *Cardamine* GT anatomy via cell cycle regulation. However, further studies will elucidate how PHB and the other HD-ZIPIII are involved in *Cardamine* GT patterning. Also, it will be interesting to understand whether HD-ZIPIII are necessary for CEM formation or whether their function is required only for formative division regulation in the CEM. Future research will shed light on the conservation of both patterning and molecular processes underlying GT variability. Whether the development of multiple cortical layers in plants is dependent on the presence of additional stem cells, CEM or both is still a matter for discussion. One possibility is that in species showing several cortical layers, the outer cortex depends on the activity of extra stem cells, whereas the inner cortical layer originates from CEM (figure 1). From this perspective, different molecular mechanisms might act in controlling cortex proliferation. On the one hand, SHR/SCR circuit might regulate additional CEI activity, hence, generating the outer layers; on the other hand, HD-ZIPIII might regulate additional divisions of the inner cortical layers. This hypothesis is also supported by recent findings showing that overexpression of *SHR* in rice leads to an increment in outer cortical layer formation [62]. Hence, it is fundamental to understand how SHR/SCR and HD-ZIPIIIs coordinate their activity to determine plant cortical layer variability. More studies on novel root monocot and dicot model system showing multiple cortical layers will permit to better elucidate the mechanisms at the basis of the variability of cortex patterning. Also, whether the knowledge acquired by studying close meristem species such as rice and *Cardamine* is applicable also in open meristem species such as pea is still completely unexplored.

Data accessibility. No original data were reported.

Authors' contributions. All of the authors were involved in the development and writing of this article.

Competing interests. We claim no competing interests.

Funding. This work was supported by an FIRB (Futuro in Ricerca 2013) project grant from the Ministero dell'Istruzione, dell'Università e della Ricerca (FIRB2013-RBFR13DCDS to G.D.R. and R.D.I.).

Acknowledgements. We acknowledge A. Rocchetti, S. Sabatini, E. Salvi, E. Pacifici, G. Bertolotti and E. Pierdonati for constructive discussions on the manuscript. We also acknowledge the referees for the valuable comments and suggestions which have led to significant improvement on the presentation and quality of this paper.

References

1. Gompel N, Prud'homme B, Wittkopp PJ, Kassner VA, Carroll SB. 2005 Chance caught on the wing: cis-regulatory evolution and the origin of pigment patterns in *Drosophila*. *Nature* **433**, 481–487. (doi:10.1038/nature03235)
2. Wittkopp PJ, Stewart EE, Arnold LL, Neidert AH, Haerum BK, Thompson EM, Akhras S, Smith-Winberry G, Shefner L. 2009 Intraspecific polymorphism to interspecific divergence: genetics of pigmentation in *Drosophila*. *Science* **326**, 540–544. (doi:10.1126/science.1176980)
3. Nikolov LA, Tsiantis M. 2017 Using mustard genomes to explore the genetic basis of evolutionary change. *Curr. Opin. Plant Biol.* **36**, 119–128. (doi:10.1016/j.pbi.2017.02.005)
4. Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B. 1993 Cellular organisation of the *Arabidopsis thaliana* root. *Development* **119**, 71–84.
5. Scheres B. 2000 Non-linear signaling for pattern formation? *Curr. Opin Plant Biol.* **3**, 412–417. (doi:10.1016/S1369-5266(00)00105-9)
6. Weigel D, Jurgens G. 2002 Stem cells that make stems. *Nature* **415**, 751–754. (doi:10.1038/415751a)
7. Steeves TA, Sussex IM. 1989 *Patterns in plant development*, 2nd edn. Cambridge, UK: Cambridge University Press.
8. Scheres B. 2007 Stem-cell niches: nursery rhymes across kingdoms. *Nat. Rev. Mol. Cell Biol.* **8**, 345–354. (doi:10.1038/nrm2164)
9. Fahn A. 1990 *Plant anatomy*, 4th edn. Oxford, UK: Pergamon.
10. Timmers AC, Auriac MC, Truchet G. 1999 Refined analysis of early symbiotic steps of the *Rhizobium–Medicago* interaction in relationship with microtubular cytoskeleton rearrangements. *Development* **126**, 3617–3628.
11. Xiao TT, Schilderink S, Moling S, Deinum EE, Kondorosi E, Franssen H, Kulikova O, Niebel A, Bisseling T. 2014 Fate map of *Medicago truncatula* root nodules. *Development* **141**, 3517–3528. (doi:10.1242/dev.110775)
12. Paungfoo-Lonhienne C *et al.* 2008 Plants can use protein as a nitrogen source without assistance from other organisms. *Proc. Natl Acad. Sci. USA* **105**, 4524–4529. (doi:10.1073/pnas.0712078105)
13. Heimsch C, Seago Jr JL. 2008 Organization of the root apical meristem in angiosperms. *Am. J. Bot.* **95**, 1–21. (doi:10.3732/ajb.95.1.1)
14. Clowes FAL. 1994 Origin of the epidermis in root meristems. *New Phytol.* **127**, 335–347. (doi:10.1111/j.1469-8137.1994.tb04284.x)
15. Pauluzzi G, Divol F, Puig J, Guiderdoni E, Dievart A, Perin C. 2012 Surfing along the root ground tissue gene network. *Dev. Biol.* **365**, 14–22. (doi:10.1016/j.ydbio.2012.02.007)
16. Kirschner GK, Stahl Y, Von Korff M, Simon R. 2017 Unique and conserved features of the barley root meristem. *Front. Plant Sci.* **8**, 1240. (doi:10.3389/fpls.2017.01240)
17. Baum SF, Dubrovsky JG, Rost TL. 2002 Apical organization and maturation of the cortex and vascular cylinder in *Arabidopsis thaliana* (Brassicaceae) roots. *Am. J. Bot.* **89**, 908–920. (doi:10.3732/ajb.89.6.908)
18. Paquette AJ, Benfey PN. 2005 Maturation of the ground tissue of the root is regulated by gibberellin and SCARECROW and requires SHORT-ROOT. *Plant Physiol.* **138**, 636–640. (doi:10.1104/pp.104.058362)
19. Clark LH, Harris WH. 1981 Observations on the root anatomy of rice (*Oryza sativa* L.). *Am. J. Botany* **68**, 154–161. (doi:10.2307/2442846)
20. Henry S, Divol F, Bettembourg M, Bureau C, Guiderdoni E, Perin C, Dievart A. 2015 Immunoprofiling of rice root cortex reveals two cortical subdomains. *Front. Plant Sci.* **6**, 1139. (doi:10.3389/fpls.2015.01139)
21. Popham RA. 1966 *Laboratory manual for plant anatomy*. Ann Arbor, MI: CV Mosby, University of Michigan.
22. Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN. 1996 The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. *Cell* **86**, 423–433. (doi:10.1016/S0092-8674(00)80115-4)
23. van den Berg C, Willemsen V, Hage W, Weisbeek P, Scheres B. 1995 Cell fate in the *Arabidopsis* root meristem determined by directional signalling. *Nature* **378**, 62–65. (doi:10.1038/378062a0)
24. Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, Sena G, Hauser MT, Benfey PN. 2000 The SHORT-ROOT gene controls radial patterning of the *Arabidopsis* root through radial signaling. *Cell* **101**, 555–567. (doi:10.1016/S0092-8674(00)80865-X)
25. Nakajima K, Sena G, Nawy T, Benfey PN. 2001 Intercellular movement of the putative transcription

factor SHR in root patterning. *Nature* **413**, 307–311. (doi:10.1038/35095061)

26. Sena G, Jung JW, Benfey PN. 2004 A broad competence to respond to SHORT ROOT revealed by tissue-specific ectopic expression. *Development* **131**, 2817–2826. (doi:10.1242/dev.01144)
27. Vaten A *et al.* 2011 Callose biosynthesis regulates symplastic trafficking during root development. *Dev. Cell* **21**, 1144–1155. (doi:10.1016/j.devcel.2011.10.006)
28. Cui H *et al.* 2007 An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. *Science* **316**, 421–425. (doi:10.1126/science.1139531)
29. Sozzani R *et al.* 2010 Spatiotemporal regulation of cell-cycle genes by SHORTROOT links patterning and growth. *Nature* **466**, 128–132. (doi:10.1038/nature09143)
30. Long Y *et al.* 2017 *In vivo* FRET-FLIM reveals cell-type-specific protein interactions in *Arabidopsis* roots. *Nature* **548**, 97–102. (doi:10.1038/nature23317)
31. Clark NM, Hinde E, Winter CM, Fisher AP, Crosti G, Blilou I, Gratton E, Benfey PN, Sozzani R. 2016 Tracking transcription factor mobility and interaction in *Arabidopsis* roots with fluorescence correlation spectroscopy. *eLife* **5**, e14770. (doi:10.7554/eLife.14770)
32. Welch D, Hassan H, Blilou I, Immink R, Heidstra R, Scheres B. 2007 Arabidopsis JACKDAW and MAGPIE zinc finger proteins delimit asymmetric cell division and stabilize tissue boundaries by restricting SHORT-ROOT action. *Genes Dev.* **21**, 2196–2204. (doi:10.1101/gad.440307)
33. Heidstra R, Welch D, Scheres B. 2004 Mosaic analyses using marked activation and deletion clones dissect *Arabidopsis* SCARECROW action in asymmetric cell division. *Genes Dev.* **18**, 1964–1969. (doi:10.1101/gad.305504)
34. Ferrell Jr JE. 2002 Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. *Curr. Opin Cell Biol.* **14**, 140–148. (doi:10.1016/S0955-0674(02)00314-9)
35. Di Mambro R *et al*. 2017 Auxin minimum triggers the developmental switch from cell division to cell differentiation in the *Arabidopsis* root. *Proc. Natl Acad. Sci. USA* **114**, E7641–E7649. (doi:10.1073/pnas.1705833114)
36. Li P *et al.* 2018 Construction of a functional casparian strip in non-endodermal lineages is orchestrated by two parallel signaling systems in *Arabidopsis thaliana*. *Curr. Biol.* **28**, 2777–2786. (doi:10.1016/j.cub.2018.07.028)
37. Moller BK, Ten Hove CA, Xiang D, Williams N, Lopez LG, Yoshida S, Smit M, Datla R, Weijers D. 2017 Auxin response cell-autonomously controls ground tissue initiation in the early *Arabidopsis* embryo. *Proc. Natl Acad. Sci. USA* **114**, E2533–E2539. (doi:10.1073/pnas.1616493114)
38. Mylona P, Linstead P, Martienssen R, Dolan L. 2002 SCHIZORIZA controls an asymmetric cell division and restricts epidermal identity in the *Arabidopsis* root. *Development* **129**, 4327–4334.
39. ten Hove CA, Willemsen V, de Vries WJ, van Dijken A, Scheres B, Heidstra R. 2010 SCHIZORIZA encodes a nuclear factor regulating asymmetry of stem cell divisions in the *Arabidopsis* root. *Curr. Biol.* **20**, 452–457. (doi:10.1016/j.cub.2010.01.018)
40. Carlsbecker A *et al.* 2010 Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* **465**, 316–321. (doi:10.1038/nature08977)
41. Miyashima S, Koi S, Hashimoto T, Nakajima K. 2011 Non-cell-autonomous microRNA165 acts in a dose-dependent manner to regulate multiple differentiation status in the *Arabidopsis* root. *Development* **138**, 2303–2313. (doi:10.1242/dev.060491)
42. Miyashima S, Honda M, Hashimoto K, Tatematsu K, Hashimoto T, Sato-Nara K, Okada K, Nakajima K. 2013 A comprehensive expression analysis of the *Arabidopsis* MICRORNA165/6 gene family during embryogenesis reveals a conserved role in meristem specification and a non-cell-autonomous function. *Plant Cell Physiol.* **54**, 375–384. (doi:10.1093/pcp/pcs188)
43. Di Ruocco G, Bertolotti G, Pacifici E, Polverari L, Tsiantis M, Sabatini S, Costantino P, Dello Ioio R. 2018 Differential spatial distribution of miR165/6 determines variability in plant root anatomy. *Development* **145**, 153858. (doi:10.1242/dev.153858)
44. Wang G, Fiers M. 2010 CLE peptide signaling during plant development. *Protoplasma* **240**, 33–43. (doi:10.1007/s00709-009-0095-y)
45. Fiers M, Golemiec E, Xu J, van der Geest L, Heidstra R, Stiekema W, Liu CM. 2005 The 14-amino acid CLV3, CLE19, and CLE40 peptides trigger consumption of the root meristem in *Arabidopsis* through a CLAVATA2-dependent pathway. *Plant Cell* **17**, 2542–2553. (doi:10.1105/tpc.105.034009)
46. Kinoshita A *et al.* 2015 A plant U-box protein, PUB4, regulates asymmetric cell division and cell proliferation in the root meristem. *Development* **142**, 444–453. (doi:10.1242/dev.113167)
47. Koizumi K, Hayashi T, Wu S, Gallagher KL. 2012 The SHORT-ROOT protein acts as a mobile, dose-dependent signal in patterning the ground tissue. *Proc. Natl Acad. Sci. USA* **109**, 13 010–13 015. (doi:10.1073/pnas.1205579109)
48. Wysocka-Diller JW, Helariutta Y, Fukaki H, Malamy JE, Benfey PN. 2000 Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot. *Development* **127**, 595–603.
49. Cui H, Benfey PN. 2009 Interplay between SCARECROW, GA and LIKE HETEROCHROMATIN PROTEIN 1 in ground tissue patterning in the *Arabidopsis* root. *Plant J.* **58**, 1016–1027. (doi:10.1111/j.1365-313X.2009.03839.x)
50. Rizzardi K, Landberg K, Nilsson L, Ljung K, Sundas-Larsson A. 2011 TFL2/LHP1 is involved in auxin biosynthesis through positive regulation of YUCCA genes. *Plant J.* **65**, 897–906. (doi:10.1111/j.1365-313X.2010.04470.x)
51. Chen W, Drapek C, Li D, Xu Z, Benfey P, Bai S. 2018 Histone deacetylase HDA19 affects cortical cell fate by interacting with SCARECROW in the *Arabidopsis* root. *bioRxiv*. (doi:10.1101/313791)
52. Heo JO, Chang KS, Kim IA, Lee MH, Lee SA, Song SK, Lee MM, Lim J. 2011 Funneling of gibberellin signaling by the GRAS transcription regulator scarecrow-like 3 in the *Arabidopsis* root. *Proc. Natl Acad. Sci. USA* **108**, 2166–2171. (doi:10.1073/pnas.1012215108)
53. Koizumi K, Gallagher KL. 2013 Identification of SHRUBBY, a SHORT-ROOT and SCARECROW interacting protein that controls root growth and radial patterning. *Development* **140**, 1292–1300. (doi:10.1242/dev.090761)
54. Cui H, Kong D, Wei P, Hao Y, Torii KU, Lee JS, Li J. 2014 SPINDLY, ERECTA, and its ligand STOMAGEN have a role in redox-mediated cortex proliferation in the *Arabidopsis* root. *Mol. Plant* **7**, 1727–1739. (doi:10.1093/mp/ssu106)
55. Jeong JS, Kim YS, Baek KH, Jung H, Ha SH, Do Choi Y, Kim M, Reuzeau C, Kim JK. 2010 Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. *Plant Physiol.* **153**, 185–197. (doi:10.1104/pp.110.154773)
56. Smith S, De Smet I. 2012 Root system architecture: insights from *Arabidopsis* and cereal crops. *Proc. R. Soc. B.* **367**, 1441–1452. (doi:10.1098/rstb.2011.0234)
57. Qin F, Kodaira KS, Maruyama K, Mizoi J, Tran LS, Fujita Y, Morimoto K, Shinozaki K, Yamaguchi-Shinozaki K. 2011 SPINDLY, a negative regulator of gibberellic acid signaling, is involved in the plant abiotic stress response. *Plant Physiol.* **157**, 1900–1913. (doi:10.1104/pp.111.187302)
58. Cui H. 2015 Cortex proliferation in the root is a protective mechanism against abiotic stress. *Plant Signal. Behav.* **10**, e1011949. (doi:10.1080/15592324.2015.1011949)
59. Coudert Y, Perin C, Courtois B, Khong NG, Gantet P. 2010 Genetic control of root development in rice, the model cereal. *Trends Plant Sci.* **15**, 219–226. (doi:10.1016/j.tplants.2010.01.008)
60. Kamiya N, Itoh J, Morikami A, Nagato Y, Matsuoka M. 2003 The SCARECROW gene's role in asymmetric cell divisions in rice plants. *Plant J.* **36**, 45–54. (doi:10.1046/j.1365-313X.2003.01856.x)
61. Wu S, Lee CM, Hayashi T, Price S, Divol F, Henry S, Pauluzzi G, Perin C, Gallagher KL. 2014 A plausible mechanism, based upon short-root movement, for regulating the number of cortex cell layers in roots. *Proc. Natl Acad. Sci. USA* **111**, 16 184–16 189. (doi:10.1073/pnas.1407371111)
62. Henry S, Dievart A, Divol F, Pauluzzi G, Meynard D, Swarup R, Wu S, Gallagher KL, Perin C. 2017 SHR overexpression induces the formation of supernumerary cell layers with cortex cell identity in rice. *Dev. Biol.* **425**, 1–7. (doi:10.1016/j.ydbio.2017.03.001)
63. Stern DL, Orgogozo V. 2009 Is genetic evolution predictable? *Science* **323**, 746–751. (doi:10.1126/science.1158997)

64. Romero IG, Ruvinsky I, Gilad Y. 2012 Comparative studies of gene expression and the evolution of gene regulation. *Nat. Rev. Genet.* **13**, 505–516. (doi:10.1038/nrg3229)
65. Mentink RA, Tsiantis M. 2015 From limbs to leaves: common themes in evolutionary diversification of organ form. *Front. Genet.* **6**, 284. (doi:10.3389/fgene.2015.00284)
66. Hay A, Barkoulas M, Tsiantis M. 2006 ASYMMETRIC LEAVES1 and auxin activities converge to repress BREVIPEDICELLUS expression and promote leaf development in *Arabidopsis*. *Development* **133**, 3955–3961. (doi:10.1242/dev.02545)
67. Barkoulas M, Hay A, Kougioumoutzi E, Tsiantis M. 2008 A developmental framework for dissected leaf formation in the *Arabidopsis* relative *Cardamine hirsuta*. *Nat. Genet.* **40**, 1136–1141. (doi:10.1038/ng.189)
68. Vlad D *et al.* 2014 Leaf shape evolution through duplication, regulatory diversification, and loss of a homeobox gene. *Science* **343**, 780–783. (doi:10.1126/science.1248384)
69. Pieper B, Monniaux M, Hay A. 2016 The genetic architecture of petal number in *Cardamine hirsuta*. *New Phytol.* **209**, 395–406. (doi:10.1111/nph.13586)
70. Kougioumoutzi E *et al.* 2013 SIMPLE LEAF3 encodes a ribosome-associated protein required for leaflet development in *Cardamine hirsuta*. *Plant J.* **73**, 533–545. (doi:10.1111/tpj.12072)
71. Rast-Somssich MI *et al*. 2015 Alternate wiring of a KNOXI genetic network underlies differences in leaf development of *A. thaliana* and *C. hirsuta*. *Genes Dev.* **29**, 2391–2404. (doi:10.1101/gad.269050.115)
72. Hofhuis H *et al*. 2016 Morphomechanical innovation drives explosive seed dispersal. *Cell* **166**, 222–233. (doi:10.1016/j.cell.2016.05.002)
73. Vuolo F, Mentink RA, Hajheidari M, Bailey CD, Filatov DA, Tsiantis M. 2017 Corrigendum: coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. *Genes Dev.* **31**, 2199. (doi:10.1101/gad.309179.117)
74. Hay AS *et al*. 2014 *Cardamine hirsuta*: a versatile genetic system for comparative studies. *Plant J.* **78**, 1–15. (doi:10.1111/tpj.12447)
75. Gan X *et al*. 2016 The *Cardamine hirsuta* genome offers insight into the evolution of morphological diversity. *Nat. Plants* **2**, 16167. (doi:10.1038/nplants.2016.167)