Review

Natural genetic variation of root system architecture from *Arabidopsis* to *Brachypodium*: towards adaptive value

David Pacheco-Villalobos and Christian S. Hardtke*

*Department of Plant Molecular Biology, University of Lausanne, Biophore Building,
1015 Lausanne, Switzerland*

Root system architecture is a trait that displays considerable plasticity because of its sensitivity to environmental stimuli. Nevertheless, to a significant degree it is genetically constrained as suggested by surveys of its natural genetic variation. A few regulators of root system architecture have been isolated as quantitative trait loci through the natural variation approach in the dicotyledon model, *Arabidopsis*. This provides proof of principle that allelic variation for root system architecture traits exists, is genetically tractable, and might be exploited for crop breeding. Beyond *Arabidopsis*, *Brachypodium* could serve as both a credible and experimentally accessible model for root system architecture variation in monocotyledons, as suggested by first glimpses of the different root morphologies of *Brachypodium* accessions. Whether a direct knowledge transfer gained from molecular model system studies will work in practice remains unclear however, because of a lack of comprehensive understanding of root system physiology in the native context. For instance, apart from a few notable exceptions, the adaptive value of genetic variation in root system modulators is unknown. Future studies should thus aim at comprehensive characterization of the role of genetic players in root system architecture variation by taking into account the native environmental conditions, in particular soil characteristics.

Keywords: root system architecture; natural genetic variation; quantitative trait locus;
Brachypodium; *Arabidopsis*

1. INTRODUCTION

Plants express a particularly high degree of phenotypic response to the environment since they are continuously exposed to fluctuating environmental conditions. This plasticity manifests itself in specific developmental, physiological and reproductive adjustments that are thought to optimize plant fitness. Excellent examples for this are the exaggerated elongation growth response of some species that allows them to escape shading by neighbouring competitors [1,2], or the control of flowering time as a function of annual or perennial lifestyle as well as climatic conditions [3]. The adaptive value of variation in these and other shoot traits can be rather obvious at times. This is often less clear when it comes to root system traits, although the root system plays a fundamental role in plant growth and survival by providing support, water and nutrients for the shoot [4,5]. Moreover, roots participate in secondary functions, including hormone biosynthesis and storage of photoassimilates. Root system architecture is considered a highly plastic trait [6,7] and is determined by developmental and environmental factors that interact to optimize exploration of the soil and the capture of

edaphic resources [8]. For example, nitrogen, phosphorus, iron and sulphur are among the nutrients that have been reported to alter post-embryonic root development and, therefore, root system architecture [9]. The modulation of root system architecture and its response to biotic and abiotic stresses is of particular interest in crop science, because it is considered that optimization of crop root systems through breeding has been largely neglected as a result of the fertilizer-intensive agriculture of the last decades [10].

2. ROOT SYSTEM ARCHITECTURE IN DICOTYLEDONS VERSUS MONOCOTYLEDONS

Root system architecture reflects the shape, three-dimensional distribution, branching pattern and age of the primary and post-embryonically generated roots [5,11]. Variation of root system architecture between different species is common, but the pronounced split between dicotyledons and monocotyledons is most prominent (figure 1). The typically allorhiz root system of dicotyledonous species consists of a single primary root and a network of lateral roots, which remain active during the plant's life cycle [5,12] (figure 1a). Here, root system architecture is mainly determined by cell division and elongation during primary and lateral root growth, as well as the extent of root branching. The system is occasionally completed by adventitious roots,

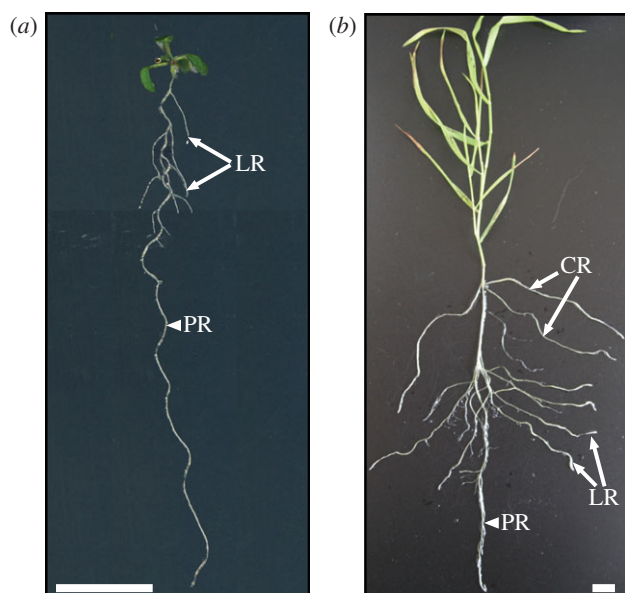*Author for correspondence (christian.hardtke@unil.ch).

Figure 1. Comparative root architecture of *Arabidopsis* and *Brachypodium*. (*a*) Typical dicotyledon allorhiz root system architecture in 10-day-old *Arabidopsis* plants. *Arabidopsis* forms only one primary root during its development, which branches out through lateral roots. (*b*) Typical monocotyledon homorhiz root system architecture in a 30-day-old *Brachypodium* plant, composed of a primary root, crown roots and lateral roots. PR, primary root; LR, lateral root; CR, crown root. Scale bars = 1 cm.

which are much more important in the typically homorhiz root systems of monocotyledons (figure 1*b*). Their root systems are characterized by a complex architecture that emerges during successive stages of development [13]. During embryogenesis, a primary root and, sometimes, a variable number of seminal roots are formed and emerge shortly after germination [13]. After the seedling stage, this system is gradually replaced by a complex shoot-borne root system that is largely composed of crown roots. While mutagenesis approaches have succeeded in the identification of a large number of loci that are essential for the making and elaboration of the root system, non-essential alleles or novel genes that modulate the architecture of functional root systems are only beginning to be identified. This is mostly through the analysis of intra-specific natural genetic variation, an approach that has been successfully applied in the dicotyledon model system, *Arabidopsis thaliana* (*Arabidopsis*).

3. NATURAL VARIATION OF ROOT SYSTEM ARCHITECTURE IN *ARABIDOPSIS*

Natural strains of *Arabidopsis*, so-called accessions, display rich variation in morphological and physiological features, including root system architecture [3,14]. This trait can be easily observed and scored in *in vitro* culture, with most studies focusing on the growth vigour of the primary root. Isolation of allelic variants that underlie observed phenotypic differences can be achieved by quantitative trait locus (QTL) mapping and subsequent map-based cloning aided by complementing, for instance, reverse genetic approaches. So far, these studies have been moderately successful in that a limited number of mostly large effect QTLs

(i.e. QTLs that explain the vast majority of the genetically determined variance in a given cross) were isolated. Notable exceptions from the large effect loci include allelic variation in a vacuolar invertase gene that explains *ca* 20 per cent of the primary root growth variance between the Cvi and Ler accessions [15], or allelic variation in the *BREVIS RADIX* (*BRX*) gene that explains *ca* 10 per cent of the variance between Eil-0 and Lc-0 [16]. However, the *BRX* gene was originally identified as a major effect QTL for root growth based on a loss-of-function allele in the Uk-1 accession [14]. Subsequent analyses implicated *BRX* in the hormonal control of primary root meristem growth [17]. In *brx* mutants, this translates into a significantly shorter primary root, more densely spaced lateral roots and more frequent adventitious roots, thus giving rise to an overall more compact and more branched adult root system. Interestingly, the later identified allele present in Lc-0 and a few other accessions encodes a hyperactive BRX protein that confers increased root growth vigour [16]. Thus, *BRX* is a rare example for an experimentally verified natural allelic series ranging from loss-of-function to hyperactive alleles when compared with other isolated QTLs, which are mostly loss-of-function only [3]. A straightforward explanation for the bias towards isolation of loss-of-function QTLs would be a focus on accessions that display the opposite extremes of a phenotype of interest. Moreover, a systematic bias towards loss-of-function alleles could be introduced by the genome evolution of *Arabidopsis*. This is suggested by first glimpses of genetic variation from comparison of accessions at the whole genome sequence level, based on re-sequencing using short read technology [18,19]. These analyses demonstrate an expected prevalence of single nucleotide polymorphisms, but also indicate a surprising number of sizeable deletions and insertions (indels) that frequently affect coding regions. It thus appears that compared with the Col-0 reference sequence, divergent accessions carry numerous null alleles, which might be prone to detection in QTL mapping [19]. This indel history of *Arabidopsis* can give rise to complicated scenarios, as exemplified by diminished root growth and eventual impaired viability arising from interaction between paralogous loci of an essential gene in amino acid biosynthesis that are located on two different chromosomes [20]. Differential evolutionary trajectories of those paralogues in Col-0 and Cvi have resulted in their differential expression and deletion of one paralogue in Cvi. The functional paralogue in Col-0 is located in the position of the deleted Cvi allele, whereas the remaining functional Cvi paralogue on a different chromosome is located in the position of the hypoactive (i.e. lowly expressed) Col-0 allele. Consequently, only certain allelic combinations produce sufficient overall protein activity, whereas other combinations impair growth rate or are not even viable.

4. *BRACHYPODIUM* AS A TRACTABLE MONOCOTYLEDON MODEL SYSTEM

Compared with dicotyledons in general and *Arabidopsis* in particular, little is known at the molecular level regarding the genetic basis of natural root system architecture

variation in grasses. In part, this might reflect the fact that genetic and molecular studies in monocotyledons typically focus on crops, mainly rice and maize [12,13,21]. Arguably, the size and generation time of these species and the growth conditions required for their rearing are limiting the throughput of genetic analyses and render these systems impractical for research groups lacking appropriate and sizeable growth facilities. The calls for an easily tractable monocotyledon model for basic research therefore appear justified and have led to the promotion of *Brachypodium distachyon* (*Brachypodium*) in this capacity, culminating in the recent publication of its draft genome [22]. *Brachypodium* belongs to the family Poaceae (the grasses), which includes the economically most important crops, notably rice, maize, wheat and barley. Diverse phylogenetic analyses have revealed that *Brachypodium* is closer to wheat and barley than rice, corn or sorghum [23,24]. Together with its many favourable characteristics as an experimental system [25], this makes *Brachypodium* a credible model species for temperate cereals and grasses, which could aid in understanding the molecular basis of important agronomic traits that are exclusively present in grass crops. Like *Arabidopsis*, *Brachypodium* possesses numerous attributes, including a small (*ca* 300 Mb) genome with a low chromosome number, diploidy in most (but not all) accessions, developed genomic resources, *Agrobacterium*-mediated transformation, small size, self-fertility and a rapid life cycle [25–28]. Moreover, its size and ease of cultivation in large numbers under simple controlled conditions make it an accessible system for smaller laboratories, which can grow *Brachypodium* in the same facilities as *Arabidopsis*, one species next to the other. So can *Brachypodium* also serve as a model system for monocotyledon root system architecture and its natural variation? The answer is yes.

5. *BRACHYPODIUM* ROOT SYSTEM MORPHOLOGY

Similar to rice and maize, *Brachypodium* develops only one primary axillary root that emerges from the base of the embryo and breaks the coleorhiza upon germination. Moreover, compared with other cereals within the Pooideae subfamily, *Brachypodium* displays a much simpler seedling root system as exemplified by the reference accession, Bd21 [29] (figure 1*b*). For example, whereas wheat typically forms three to five primary roots, *Brachypodium* has only one and is in this sense more similar to rice or maize [27–29]. However, at later, adult stages of development *Brachypodium* and wheat display very similar root systems, which are dominated by branch roots [29]. At the tissue level, cellular organization is more complex in *Brachypodium* than in *Arabidopsis* with respect to cell types and numbers. For instance, *Brachypodium* has several layers with a variable number of cortical cells, whereas *Arabidopsis* has only one layer with a fixed number of cells. However, the cellular organization of *Brachypodium* roots seems to be less complex than that of other cereals in terms of the number of cells and layers in each tissue, which facilitates histological analysis in this grass species. Cross sections of *Brachypodium* primary roots show a single layer of epidermal cells, ground tissue consisting of five cortical and one endodermal cell layer, and the vasculature (figure 2*a*). The central vascular cylinder consists of one or sometimes two large central metaxylem tracheary element(s) and six peripheral xylem tracheary elements. The latter are alternating with phloem, which is typical of monocotyledon species. The phloem is composed of a protophloem sieve tube associated with two companion cells and the metaphloem sieve element and is organized in a symmetrical pattern. Finally, the external cell layer of the central cylinder, the pericycle, inter-crosses with protoxylem vessels at the sites of peripheral xylem bundles (figure 2*b*).

The principles of tissue layer development of *Brachypodium* primary roots appear to be similar to those previously described for rice [19,29] (figure 2*b–e*). First, a group of initials divides and differentiates into columella cells as well as peripheral root cap cells. Second, the epidermis, the endodermis and all the cortical cell layers develop from another group of initials, termed epidermis–endodermis initials, through successive asymmetric periclinal divisions. In rice, it is well documented that the number of such periclinal divisions varies between different root types, explaining the differences in the layers of cortical cells [30]. The cortical origin of epidermis is a shared feature among monocotyledons and differs from dicotyledons, where epidermal and lateral root cap cells originate from a common initial [31]. Finally, the stele initials, which are larger than the surrounding meristematic cells, undergo several divisions to produce precursor cells that will differentiate into the vascular tissues.

6. *BRACHYPODIUM* AS A MODEL FOR NATURAL VARIATION OF ROOT SYSTEM ARCHITECTURE IN MONOCOTYLEDONS

Similar to *Arabidopsis*, a collection of natural accessions collected from a wide range of geographical locations and derived inbred lines are currently being established for *Brachypodium* [32,33]. Phenotypic variation has already been found for diverse traits in a collection of Turkish accessions, indicating that this germplasm is suitable for natural variation analysis [32,34]. For example, substantial variation in flowering and vernalization time among *Brachypodium* accessions has been reported in one study [35], whereas broad diversity in drought tolerance was found in another [36]. Natural variation of *Brachypodium* root system architecture has not been investigated yet. However, a preliminary assessment of phenotypic variation in root growth in our laboratory, which monitored 178 diploid accessions from Turkey [32,34] and the community standard Bd21, looks promising (figure 3*a,b*). To observe *Brachypodium* root growth, seeds can be germinated in vertically oriented agar plates on standard media (half strength Murashige & Skoog (MS) nutrients, 1% sucrose), because they remain attached to the media surface very easily owing to their shape and small size. Depending on the growth conditions, up to 32 seedlings can be maintained on 10 × 10 cm square plates up to 10 days after germination (dag). Moreover, lateral root growth can be followed by using large square plates (24 × 24 cm), which can
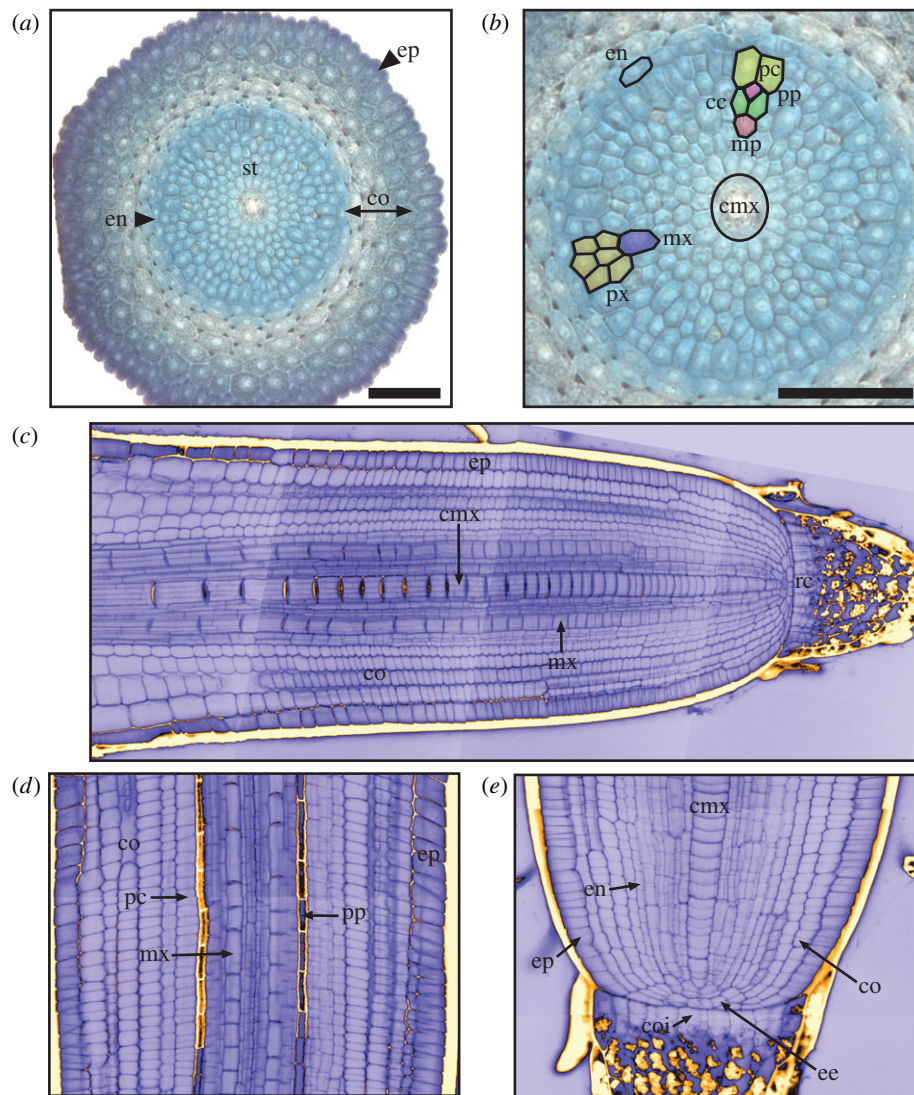
Figure 2. Cellular structure of the *Brachypodium* primary root. (*a*) Transverse section across a *Brachypodium* primary root at meristem level (*ca* 300 μm from the root tip) stained with toluidine blue in light microscopy. (*b*) Magnified view of the stele from the same transverse section. Vascular tissues are organized in a polyarch structure containing numerous xylem vessels alternating with phloem sieve tubes. One central metaxylem element is found in the centre of the stele, surrounded by several smaller metaxylem vessels. The phloem presents a characteristic symmetrical pattern in which protophloem is associated with two companion cells and one metaphloem cell. Scale bars in (*a,b*) = 50 μm. (*c*) Longitudinal view of a primary root stained by the mPS-PI procedure and visualized by confocal microscopy. (*d*) Detailed view of the vascular tissues in the stele of the same mPS-PI-stained root. Note the characteristic staining of the protophloem cell files owing to the cell wall thickening that occurs during its differentiation. (*e*) Detailed view of the organization of initials at the root apical meristem. The tip of the apical meristem is occupied by a quiescent centre. Columella initials are generated through an anticlinal division. The epidermis–endodermis initials undergo a first anticlinal division near the quiescent centre. The endodermis and cortex cells are produced by successive periclinal divisions. ep, epidermis; co, cortex; en, endodermis; st, stele; pc, pericycle; pp, protophloem; cc, companion cell; px, protoxylem; mx, metaxylem; cmx, central metaxylem; coi, columella initial; ee, epidermis–endodermis initial; qc, quiescent centre.

accommodate *Brachypodium* plants up to 25 dag, or by using tubes that can be adapted for new phenotype imaging methods (figure 3*c*) [37,38]. Finally, whole mount examination of cellular structure is possible in *Brachypodium* by using the modified pseudo-Schiff propidium iodide (mPS-PI) staining method combined with confocal laser scanning microscopy (figure 2*c*−*e*) [39]. With this set-up, differences in quantitative cellular parameters were found among accessions collected from eight different principal locations, indicating phenotypic diversity and the potential of this germplasm collection to reveal QTLs involved in primary root development (figure 3*b*). Thus, *Brachypodium* accessions could provide a

valuable resource, enabling us to understand monocotyledon root system architecture plasticity and thereby increase our knowledge to breed grasses that can exploit soil resources more efficiently.

7. NATURAL VARIATION OF ROOT SYSTEM ARCHITECTURE—PINNING DOWN THE ADAPTIVE VALUE

In summary, abundant natural variation in root system architecture exists in both monocotyledons and dicotyledons, and its genetic basis is beginning to be unravelled. A major question relating to this variation
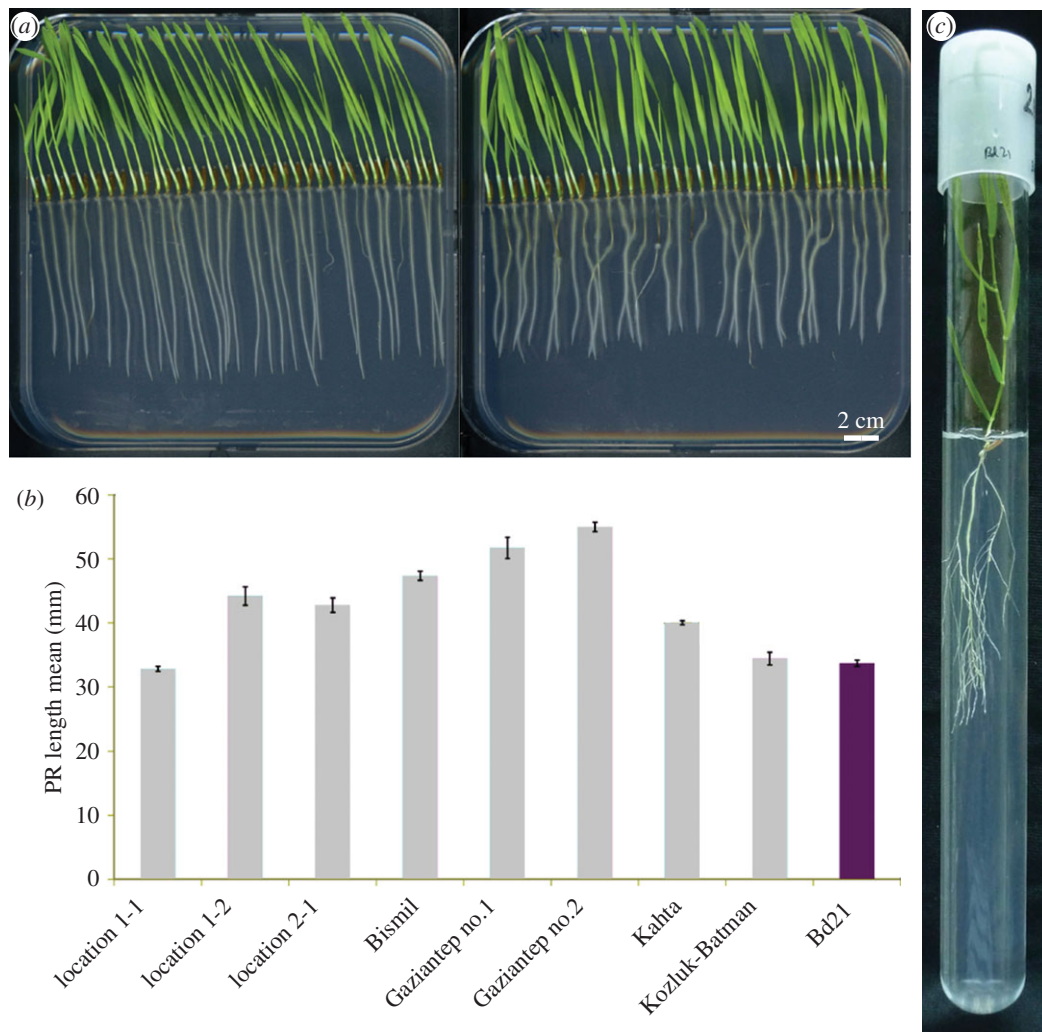
Figure 3. *Brachypodium* as a model for natural variation analysis of root system growth and architecture. (*a*) Two different *Brachypodium* accessions from Turkey. Note the difference in primary root length. Seeds were germinated in half strength MS-agar plates and incubated in vertical position in continuous light for 6 days. (*b*) Primary root length measurement of 6-day-old *Brachypodium* accessions from eight different locations in Turkey. The primary root length of the standard community accession Bd21 is also shown. Note the differences in root length among the locations. Vertical lines indicate standard error. (*c*) Bd21 plant after 30 days of growth in a tube containing half strength MS-phytagel under continuous light. The seed was germinated directly in the tube.

has however hardly been addressed: what is the adaptive value of different root system morphologies? Against a background of efforts aiming towards a more sustainable agriculture, there is a general notion that the impact of the root system on plant performance has been largely neglected in crop breeding. The hopes for a positive impact of root system architecture on shoot performance in agriculture are high and has led to calls for a 'Second Green Revolution', which should focus on root system architecture and should be made 'a priority for plant biology in the twenty-first century' [40]. Indeed, root system architecture critically influences nutrient and water uptake efficiency [41,42]. For example, rooting depth impacts the efficient acquisition of soil nitrogen (and water) since nitrate leeches down the soil profile, while manipulating the root growth angle could lead to more efficient use of phosphate, which typically accumulates in top soil [43]. Although it appears that root system size generally does not limit the acquisition of mobile macronutrients [44], such as nitrate, exploration of a larger soil volume by the root system could be

advantageous when immobile macronutrients, such as phosphate, or water availability are limiting growth, in particular in competitive situations [42].

However, beyond these general notions, surprisingly few studies address the topic of the adaptive value of root system architecture directly and in a genetically tractable manner. An example is a study that has conclusively demonstrated the effect of root morphology on nutrient uptake by comparing an *Arabidopsis* mutant with significantly decreased levels of root branching to its wild-type background under nutrient stress [45]. Other studies have investigated the importance of the root system for shoot performance in a competitive situation. For instance, for variation at the *BRX* locus, a conditional fitness effect of the root system on shoot performance depending on underground competition was demonstrated [46]. Such studies could be extended to involve more genotypes, clarifying, for instance, whether a mix of different genetically determined root system architectures within a population improves the collective soil exploration efficiency and could thus be maintained

by frequency-dependent selection. However, to get to the root (*sic!*) of the adaptive value of root system architecture variation, future studies should focus on investigating genotypes of interest in the light of their ecological background. A prime example is the isolation of allelic variation in the expression levels of a sodium transporter in the root and thus salt accumulation in the shoot, which could be associated with beach versus inland habitats and suggests a clear adaptation to increased salt levels in a coastal cline [47,48]. Taking into account the soil environment is maybe the most important factor and is becoming feasible as numerous recently collected accessions (both of *Arabidopsis* and *Brachypodium*) from precisely mapped collection sites are becoming available. Finally, interactions with micro-organisms of the rhizosphere are likely to impact the *in situ* relevance of root system architecture for plant performance. Genetically tractable analysis of these environment × genotype interactions, and thereby evaluation of the adaptive value of root system architecture and the corresponding role of individual alleles, can probably only be resolved in field trial settings that involve transgenic plants.

REFERENCES

1 Kami, C., Lorrain, S., Hornitschek, P. & Fankhauser, C. 2010 Light-regulated plant growth and development. *Curr. Top. Dev. Biol.* **91**, 29–66. (doi:10.1016/S0070-2153(10)91002-8)

2 Kebrom, T. H. & Brutnell, T. P. 2007 The molecular analysis of the shade avoidance syndrome in the grasses has begun. *J. Exp. Bot.* **58**, 3079–3089. (doi:10.1093/jxb/erm205)

3 Alonso-Blanco, C., Aarts, M. G., Bentsink, L., Keurentjes, J. J., Reymond, M., Vreugdenhil, D. & Koornneef, M. 2009 What has natural variation taught us about plant development, physiology, and adaptation? *Plant Cell* **21**, 1877–1896. (doi:10.1105/tpc.109.068114)

4 Aiken, R. M. & Smucker, A. J. 1996 Root system regulation of whole plant growth. *Annu. Rev. Phytopathol.* **34**, 325–346. (doi:10.1146/annurev.phyto.34.1.325)

5 Osmont, K. S., Sibout, R. & Hardtke, C. S. 2007 Hidden branches: developments in root system architecture. *Annu. Rev. Plant Biol.* **58**, 93–113. (doi:10.1146/annurev.arplant.58.032806.104006)

6 Potters, G., Pasternak, T. P., Guisez, Y. & Jansen, M. A. 2009 Different stresses, similar morphogenic responses: integrating a plethora of pathways. *Plant Cell Environ.* **32**, 158–169. (doi:10.1111/j.1365-3040.2008.01908.x)

7 Potters, G., Pasternak, T. P., Guisez, Y., Palme, K. J. & Jansen, M. A. 2007 Stress-induced morphogenic responses: growing out of trouble? *Trends Plant Sci.* **12**, 98–105. (doi:10.1016/j.tplants.2007.01.004)

8 Malamy, J. E. 2005 Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell Environ.* **28**, 67–77. (doi:10.1111/j.1365-3040.2005.01306.x)

9 Lopez-Bucio, J., Cruz-Ramirez, A. & Herrera-Estrella, L. 2003 The role of nutrient availability in regulating root architecture. *Curr. Opin. Plant Biol.* **6**, 280–287. (doi:10.1016/S1369-5266(03)00035-9)

10 Bevan, M. W., Garvin, D. F. & Vogel, J. P. 2010 *Brachypodium distachyon* genomics for sustainable food and fuel production. *Curr. Opin. Biotechnol.* **21**, 211–217. (doi:10.1016/j.copbio.2010.03.006)

11 Lynch, J. 1995 Root architecture and plant productivity. *Plant Physiol.* **109**, 7–13.

12 Hochholdinger, F. & Zimmermann, R. 2008 Conserved and diverse mechanisms in root development. *Curr. Opin. Plant Biol.* **11**, 70–74. (doi:10.1016/j.pbi.2007.10.002)

13 Hochholdinger, F. & Tuberosa, R. 2009 Genetic and genomic dissection of maize root development and architecture. *Curr. Opin. Plant Biol.* **12**, 172–177. (doi:10.1016/j.pbi.2008.12.002)

14 Mouchel, C. F., Briggs, G. C. & Hardtke, C. S. 2004 Natural genetic variation in *Arabidopsis* identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root. *Genes Dev.* **18**, 700–714. (doi:10.1101/gad.1187704)

15 Sergeeva, L. I., Keurentjes, J. J., Bentsink, L., Vonk, J., van der Plas, L. H., Koornneef, M. & Vreugdenhil, D. 2006 Vacuolar invertase regulates elongation of *Arabidopsis thaliana* roots as revealed by QTL and mutant analysis. *Proc. Natl Acad. Sci. USA* **103**, 2994–2999. (doi:10.1073/pnas.0511015103)

16 Beuchat, J., Li, S., Ragni, L., Shindo, C., Kohn, M. H. & Hardtke, C. S. 2010 A hyperactive quantitative trait locus allele of *Arabidopsis* BRX contributes to natural variation in root growth vigor. *Proc. Natl Acad. Sci. USA* **107**, 8475–8480. (doi:10.1073/pnas.0913207107)

17 Scacchi, E., Salinas, P., Gujas, B., Santuari, L., Krogan, N., Ragni, L., Berleth, T. & Hardtke, C. S. 2010 Spatio-temporal sequence of cross-regulatory events in root meristem growth. *Proc. Natl Acad. Sci. USA* **107**, 22 734–22 739. (doi:10.1073/pnas.1014716108)

18 Ossowski, S., Schneeberger, K., Clark, R. M., Lanz, C., Warthmann, N. & Weigel, D. 2008 Sequencing of natural strains of *Arabidopsis thaliana* with short reads. *Genome Res.* **18**, 2024–2033. (doi:10.1101/gr.080200.108)

19 Santuari, L., Pradervand, S., Amiguet-Vercher, A. M., Thomas, J., Dorcey, E., Harshman, K., Xenarios, I., Juenger, T. E. & Hardtke, C. S. 2010 Substantial deletion overlap among divergent *Arabidopsis* genomes revealed by intersection of short reads and tiling arrays. *Genome Biol.* **11**, R4. (doi:10.1186/gb-2010-11-1-r4)

20 Bikard, D., Patel, D., Le Mette, C., Giorgi, V., Camilleri, C., Bennett, M. J. & Loudet, O. 2009 Divergent evolution of duplicate genes leads to genetic incompatibilities within *A. thaliana*. *Science* **323**, 623–626. (doi:10.1126/science.1165917)

21 Coudert, Y., Perin, C., Courtois, B., Khong, N. G. & 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)

22 The International Brachypodium Initiative. 2010 Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature* **463**, 763–768. (doi:10.1038/nature08747)

23 Kellogg, E. A. 2001 Evolutionary history of the grasses. *Plant Physiol.* **125**, 1198–1205. (doi:10.1104/pp.125.3.1198)

24 Vogel, J. P. *et al.* 2006 EST sequencing and phylogenetic analysis of the model grass *Brachypodium distachyon*. *Theor. Appl. Genet.* **113**, 186–195. (doi:10.1007/s00122-006-0285-3)

25 Draper, J. *et al.* 2001 *Brachypodium distachyon*: a new model system for functional genomics in grasses. *Plant Physiol.* **127**, 1539–1555. (doi:10.1104/pp.010196)

26 Brkljacic, J. *et al.* 2011 *Brachypodium* as a model for the grasses: today and the future. *Plant Physiol.* **157**, 3–13. (doi:10.1104/pp.111.179531)

27 Vogel, J. & Bragg, J. 2009 *Brachypodium distachyon*, a new model for the Triticeae. In *Genetics and genomics of the Triticeae, plant genetics and genomics: crops and models* (eds C. Feuillet & G. J. Muehlbauer), pp. 427–449. New York, NY: Springer.

28 Garvin, D. F. *et al.* 2008 Development of genetic and genomic research resources for *Brachypodium distachyon*, a new model system for grass crop research. *Crop Sci.* **48**, S69–S84. (doi:10.2135/cropsci2007.04.0208)

29 Watt, M., Schneebeli, K., Dong, P. & Wilson, W. I. 2009 The shoot and root growth of *Brachypodium* and its potential as a model for wheat and other cereal crops. *Funct. Plant Biol.* **36**, 960–969. (doi:10.1071/FP09214)

30 Rebouillat, J. *et al.* 2009 Molecular genetics of rice root development. *Rice* **2**, 15–34. (doi:10.1007/s12284-008-9016-5)

31 Clowes, F. A. L. 1994 Origin of the epidermis in root meristems. *New Phytol.* **127**, 335–347. (doi:10.1111/j.1469-8137.1994.tb04284.x)

32 Vogel, J. P., Tuna, M., Budak, H., Huo, N., Gu, Y. Q. & Steinwand, M. A. 2009 Development of SSR markers and analysis of diversity in Turkish populations of *Brachypodium distachyon*. *BMC Plant Biol.* **9**, 88. (doi:10.1186/1471-2229-9-88)

33 Mur, L. A., Allainguillaume, J., Catalan, P., Hasterok, R., Jenkins, G., Lesniewska, K., Thomas, I. & Vogel, J. 2011 Exploiting the *Brachypodium* Tool Box in cereal and grass research. *New Phytol.* **191**, 334–347. (doi:10.1111/j.1469-8137.2011.03748.x)

34 Filiz, E., Ozdemir, B. S., Budak, F., Vogel, J. P., Tuna, M. & Budak, H. 2009 Molecular, morphological, and cytological analysis of diverse *Brachypodium distachyon* inbred lines. *Genome* **52**, 876–890. (doi:10.1139/G09-062)

35 Schwartz, C. J., Doyle, M. R., Manzaneda, A. J., Rey, P. J., Mitchell-Olds, T. & Amasino, R. M. 2011 Natural variation of flowering time and vernalization responsiveness in *Brachypodium distachyon*. *Bioener. Res.* **3**, 38–46. (doi:10.1007/s12155-009-9069-3)

36 Luo, N., Liu, J., Yu, X. & Jiang, Y. 2011 Natural variation of drought response in *Brachypodium distachyon*. *Physiol. Plant* **141**, 19–29. (doi:10.1111/j.1399-3054.2010.01413.x)

37 Clark, R. T., MacCurdy, R. B., Jung, J. K., Shaff, J. E., McCouch, S. R., Aneshansley, D. J. & Kochian, L. V. 2011 Three-dimensional root phenotyping with a novel imaging and software platform. *Plant Physiol.* **156**, 455–465. (doi:10.1104/pp.110.169102)

38 Iyer-Pascuzzi, A. S., Symonova, O., Mileyko, Y., Hao, Y., Belcher, H., Harer, J., Weitz, J. S. & Benfey, P. N. 2010 Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. *Plant Physiol.* **152**, 1148–1157. (doi:10.1104/pp.109.150748)

39 Truernit, E., Bauby, H., Dubreucq, B., Grandjean, O., Runions, J., Barthelemy, J. & Palauqui, J. C. 2008 High-resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of phloem development and structure in *Arabidopsis*. *Plant Cell* **20**, 1494–1503. (doi:10.1105/tpc.107.056069)

40 Lynch, J. P. 2007 Roots of the second green revolution. *Aust. J. Bot.* **55**, 493–512. (doi:10.1071/BT06118)

41 Linkohr, B. I., Williamson, L. C., Fitter, A. H. & Leyser, H. M. 2002 Nitrate and phosphate availability and distribution have different effects on root system architecture of *Arabidopsis*. *Plant J.* **29**, 751–760. (doi:10.1046/j.1365-313X.2002.01251.x)

42 Schenk, H. J. 2006 Root competition: beyond resource depletion. *J. Ecol.* **94**, 725–739. (doi:10.1111/j.1365-2745.2006.01124.x)

43 Ge, Z., Rubio, G. & Lynch, J. P. 2000 The importance of root gravitropism for inter-root competition and phosphorus acquisition efficiency: results from a geometric simulation model. *Plant Soil* **218**, 159–171. (doi:10.1023/A:1014987710937)

44 Robinson, D. 1996 Resource capture by localized root proliferation: why do plants bother? *Ann. Bot. Lond.* **77**, 179–185. (doi:10.1006/anbo.1996.0020)

45 Fitter, A., Williamson, L., Linkohr, B. & Leyser, O. 2002 Root system architecture determines fitness in an *Arabidopsis* mutant in competition for immobile phosphate ions but not for nitrate ions. *Proc. R. Soc. Lond. B* **269**, 2017–2022. (doi:10.1098/rspb.2002.2120)

46 Shindo, C., Bernasconi, G. & Hardtke, C. S. 2008 Intraspecific competition reveals conditional fitness effects of single gene polymorphism at the *Arabidopsis* root growth regulator *BRX*. *New Phytol.* **180**, 71–80. (doi:10.1111/j.1469-8137.2008.02553.x)

47 Rus, A., Baxter, I., Muthukumar, B., Gustin, J., Lahner, B., Yakubova, E. & Salt, D. E. 2006 Natural variants of AtHKT1 enhance Na$^+$ accumulation in two wild populations of *Arabidopsis*. *PLoS Genet.* **2**, e210. (doi:10.1371/journal.pgen.0020210)

48 Baxter, I. *et al.* 2010 A coastal cline in sodium accumulation in *Arabidopsis thaliana* is driven by natural variation of the sodium transporter AtHKT1;1. *PLoS Genet.* **6**, e1001193. (doi:10.1371/journal.pgen.1001193)