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Review

Looking for Root Hairs to Overcome Poor Soils

Thanyakorn Rongsawat,[1,2] Jean-Benoît Peltier,[1] Jean-Christophe Boyer,[1] Anne-Aliénor Véry,[1] and Hervé Sentenac[1,*]

Breeding new cultivars allowing reduced fertilization and irrigation is a major challenge. International efforts towards this goal focus on noninvasive methodologies, platforms for high-throughput phenotyping of large plant populations, and quantitative description of root traits as predictors of crop performance in environments with limited water and nutrient availability. However, these high-throughput analyses ignore one crucial component of the root system: root hairs (RHs). Here, we review current knowledge on RH functions, mainly in the context of plant hydromineral nutrition, and take stock of quantitative genetics data pointing at correlations between RH traits and plant biomass production and yield components.

Highlights

Plant breeding for improved belowground traits, allowing reduced fertilization and irrigation inputs, can contribute to the development of sustainable agriculture practices.

Root hairs (RHs) increase the volume of exploited soil, and have major roles in nutrient and water uptake as well as in beneficial interactions with soil microorganisms.

Plant engagement in mycorrhizal symbiosis also increases the volume of exploited soil, but appears less efficient than RH development in terms of biomass production in some soil conditions.

Evidence that plant biomass production can be positively correlated to RH length is available.

Root Hairs Enlarge the Soil–Root Interface

Research efforts aiming at improving understanding of the functioning of root systems are required to better exploit the genetic variation in productivity of crops in poorly fertile soils and to develop new crop cultivars with enhanced capacity for soil resource acquisition [1,2]. Root system architecture and, at the root–soil interface, production and elongation of root hairs (RHs), are major determinants of the location and volume of exploited soil, which is why RHs take center stage in this review. It has been reported that a single rye plant (*Secale cereale*) can develop more than 10^{10} RHs, representing an underground interface of ~400 m^2, much larger than that of the aerial parts of the plant [3]. Figure 1 (Key Figure) shows dense and long RHs over almost the whole root system in a 2-week old wheat (*Triticum turgidum* ssp. *durum*) seedling. The diameter of the RH cylinder around the root in the displayed enlargement is approximately ten times larger than that of the root itself and, thus, the volume of this cylinder would be *ca.* 100 times larger than that of the root. Such a figure indicates that the ability of the root system to take up poorly mobile nutrient ions (e.g., phosphate; see later) can be significantly increased by RH production. Here, we review some major functions of RHs, in the context of plant mineral nutrition, and scrutinize recent attempts to use RH traits in plant breeding programs.

Adhesion to Soil Particles, Soil Penetration, and Rhizosheath Formation

RHs enhance seedling survival upon soil disruption by favoring root anchoring [4]. They also provide grip for root tip penetration in soil. For instance, during germination of maize on a soil displaying a moderate penetrometer resistance, wild-type seedlings took ~16 h to anchor themselves to the soil, compared with >30 h for hairless mutant seedlings, most of which did not become anchored securely [5]. The strength of the grip can be increased by root exudation of adhering molecules [6], as also shown in clinging-climber species, such as English ivy, and their specialized RHs [7]. However, when the resistance to vertical uprooting forces is compared between arabidopsis (*Arabidopsis thaliana*) wild-type plants and mutant plants impaired in RH development or lateral root production, the conclusion was that RHs do not contribute to whole-plant anchoring in this operational definition [8].

At a later stage of root system development, RHs and root exudation of adhesive molecules are involved in **rhizosheath** (see Glossary) formation [6], which contributes to plant adaptation to abiotic and biotic conditions, as prevention of water loss, nutrient and water acquisition, and

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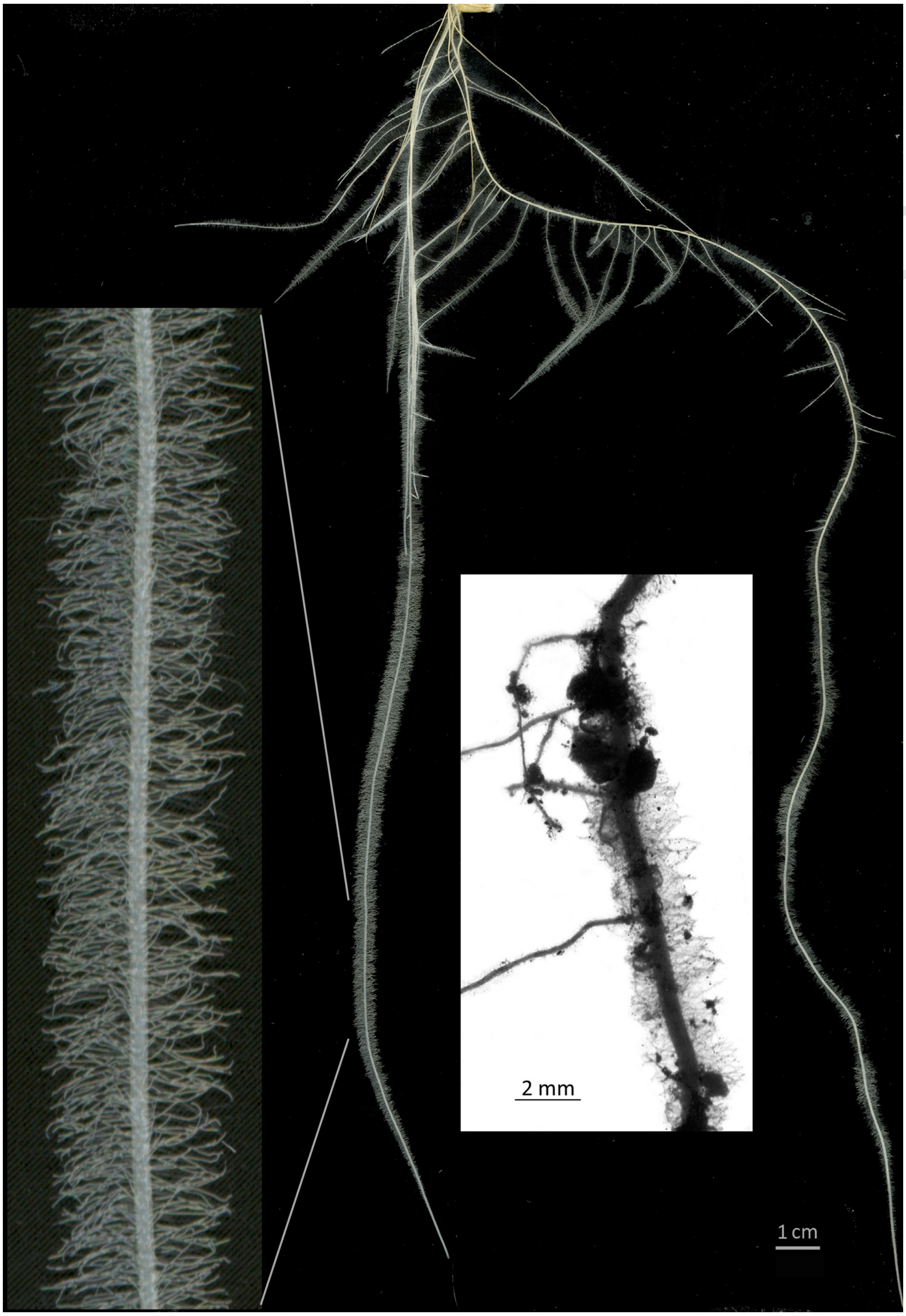
Key Figure

Q1

Root System of Wheat Seedlings



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Glossary

Rhizosheath: root–soil sheath resulting from agglutination and adhesion to the root of soil particles. Operational descriptions are proposed from the weight of soil that adheres to roots that have been freshly excavated and submitted to a soil removal treatment (e.g., hand shaking or sonication in water) of standardized vigor [9,11,12].

Shaker channels: highly K^+-selective voltage-gated channels. The plant Shaker family comprises both hyperpolarization-activated and depolarization-activated channels, involved in K^+ uptake or K^+ secretion, respectively. The name 'Shaker' comes from the phenotype of a fly (*Drosophila melanogaster*) mutant characterized by rapid abnormal movements, in which the first channel of this family was cloned a few years before the cloning of channels of the same type in plants.

Transceptor: acronym of 'transporter' and 'receptor'. A transceptor is endowed with the capacity to mediate membrane transport and the ability to sense and signal the availability of a given solute.

mechanical defense against herbivorous and plant parasites [9]. A strong correlation has been found between RH length and rhizosheath weight in wheat [10]. The correlation found in barley (*Hordeum vulgare*) is weaker [11], and no significant correlation has been observed in 58 other species except for those with quite short RHs [12]. It has been proposed that, when RH length exceeds ~300 µm, other factors have increasing importance in rhizosheath size and stability, which might include RH density and RH morphology (e.g., bent or hooked forms that would trap more soil, and root and microbial mucilage) [9,12].

Nutrient and Water Acquisition

Major lines of evidence that RHs contribute significantly to nutrient ion acquisition from the soil can be sorted as follows: (i) nutrient starvation results in increased RH density and length [13,14]; (ii) mutant plants displaying impaired RH growth show poor nutrient ion uptake and biomass production; furthermore, nutrient accumulation is positively correlated with RH length under nutrient-deficient conditions [15–18]; (iii) genotypes with longer RHs have been shown in barley and wheat to be better adapted to low nutrient soil [19,20]; and (iv) evidence that RHs contribute directly to nutrient uptake has been obtained by various electrophysiological approaches [21–23] or by using dedicated growth devices ensuring that only RHs had access to the nutrient source [24].

Evidence has also been obtained that RHs can facilitate water uptake [18,25,26]. For instance, the absence of RHs affects water absorption and drought tolerance in arabidopsis [18]. In barley, analyses of the relationship between transpiration rate and xylem suction in wild type and hairless mutant plants provided direct evidence that RHs contribute to water uptake in drying soils in rapidly transpiring plants by increasing the soil–root interface [26]. RHs are also involved in the formation of rhizosheaths, which are more developed in mesophytic grasses in drier conditions [9], which also supports the hypothesis that the control of RH development has a role in plant adaptation to drought conditions.

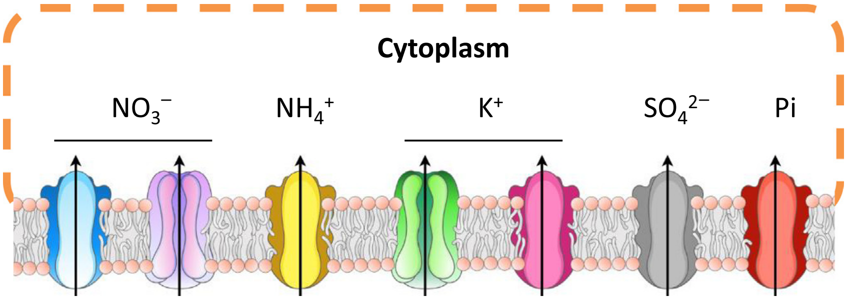
Ion Transport Systems at the RH Plasma Membrane

Molecular analyses, including reverse genetics approaches, most often carried out in arabidopsis, have provided information on the transporters and channels involved in nutrient ion acquisition by roots. Here, we summarize our current understanding regarding potassium (K), nitrogen (N), phosphorus (P), and sulfur (S) acquisition (Figure 2).

K^+ uptake from the soil by arabidopsis roots is essentially mediated by the high-affinity K^+ transporter AtHAK5 and the **Shaker channels** AtAKT1 and AtKC1 [27]. Evidence is available that these three K^+ transport systems are expressed in RHs [15,28].

NO_3^- acquisition by roots involves transporters belonging to three different families: nitrate transporter 1/peptide transporter family (NPF), NRT2, and NRT3 (also named NAR2 for 'nitrate assimilation related family') [29]. The NPF family comprises the extensively studied AtNPF6.3 '**transceptor**' (endowed with a dual NO_3^- transport/signaling function [30]), initially named CHL1 or AtNRT1.1. This membrane protein behaves both as a dual-affinity NO_3^- transporter and as a NO_3^- sensor mediating NO_3^- regulated auxin transport, thereby having an important role in root development [29,31]. Transcriptome data provide evidence that *AtNPF6.3/AtNRT1.1* transcripts are present in arabidopsis RHs [32]. The NRT2 family comprises AtNRT2.1 and AtNRT2.2, which physically interact with a member of the NRT3 family, AtNRT3.1 (also named NAR2.1) to form

Figure 1. Main photo: 2-week-old seedling grown in a rhizobox. Inset: part of the root system of a plant grown for 2 months in soil in a pot, showing root hairs in old parts of the root system. Wheat cultivar: Oued Zenati.



Family name	NRT2-NRT3 complexes	NPF6	AMT	Shaker	HAK	Sultr	PHT
Major systems	NRT2.1 and NRT3.1	NPF6.3 also named CHL1 or NRT1	AMT1;1 AMT1;2 AMT1;3	AKT1 KC1	HAK5	SULTR1;1 SULTR1;2	PHT1;1 to 1;4
References	[29,32]	[29,32-34]	[32,35]	[27,28]	[15,27]	[32,39]	[37]

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Figure 2. Ion Channels and Transporters Involved in Nutrient Ion Uptake and to Display Expression in Root Hairs in *Arabidopsis thaliana*. Root hair transcriptome data obtained in *Medicago truncatula*, *Glycine max*, *Solanum lycopersicum*, *Zea mays*, and *Cucumis sativus* indicate that close homologs of these arabidopsis channels and transporters are also expressed in root hairs of these dicots and monocots, as well as in those of the lycophyte *Selaginella moellendorffii* [32,40]. The expression of genes encoding high-affinity uptake systems can be repressed in rich media, which might be why expression in root hairs of genes such as *AtHAK5* and *AtNRT2;1* is not always revealed by transcriptome analyses of plants grown on rich media while evidenced by other studies using more diluted media (e.g., [15,34]). See [15,27–29,32–35,37,39].

heteromeric structures that contribute significantly to high-affinity NO_3^- uptake from the soil solution [29,33]. *AtNRT2.1* and *AtNRT3.1* display expression in RHs as shown by reporter gene experiments and/or transcriptome analyses [32,34]. RHs also express members of the AMT, PHT1, and Sultr families, involved in ammonium [35], phosphate [36–38], and sulfate [39] uptake in arabidopsis, respectively [32].

Close homologs of all the above-cited channels and transporters, (*AtAKT1, AtKC1, AtHAK5, NPF6.3, AtNRT2.1*, and *AtNRT3.1*) as well as ammonium, phosphate, and sulfate transporters from the AMT, PHT1, and Sultr families, have been identified in *Medicago truncatula* RH transcriptome data by analyses focused on membrane transport systems [40], and can be found in other RH transcriptomes from both dicots and monocots [32]. Shaker channels and members of the HAK, NRT2, NRT3, AMT, PHT, and Sultr transporter families are also present in the moss *Physcomitrella patens* [41–44][i]. Altogether, these observations suggest that major components of the RH equipment involved in plant mineral nutrition were acquired very early during plant evolution.

The sensitivity of RH length and density to nutrient and water availability in the soil (see earlier) may involve a role of RHs as sensors of soil conditions. Such a hypothesis of early sensing of water deficit conditions has received support from studies in barley wild type and hairless mutant plants. Analysis of transcriptomes from roots that were sampled at the onset of a water stress revealed that more genes were induced in the roots of the wild-type plants, including, for example, genes involved in abscisic acid biosynthesis [45]. In terms of nutrient sensing, the arabidopsis NO_3^- transporter AtNPF6.3/AtNRT1.1 and the Shaker K^+ channel AtAKT1, which have both been proposed to behave as transceptors, able to sense and signal the availability of their substrates, NO_3^- and K^+, respectively [30,46], are both expressed in RHs (see earlier).

RH Production versus Engagement in Mycorrhizal Symbiosis

Both RH production and engagement in mycorrhizal symbiosis result in increased soil exploration and exploitation. Arbuscular endomycorrhizal colonization (AM) is associated with either a decrease [47,48] or an increase [49] in RH density and length, depending in some species on the root type (lateral root order) [49]. The decrease has been proposed to result from changes in the root metabolic status and competition for available photosynthates between RH production and the fungus [47]. The increase has been associated with changes in the expression of auxin metabolism and transport genes, which are likely to impact RH development [50] (see later). In ectomycorrhizal symbiosis, evidence is available that fungal secretion of the auxin antagonist hypaphorine can inhibit RH development [51,52].

Mycorrhizal fungal hyphae can have a smaller diameter (ca. 4 μm for *Glomus intraradices* and 5 μm for *Glomus mosseae* [53]) compared with RHs (ca. 10 μm in arabidopsis [54]), which allows exploration of smaller soil pores. Furthermore, they extend far beyond the limits of the RH cylinder. Thus, mycorrhizal symbiosis may be hypothesized to be more efficient for exploiting the soil compared with the promotion of RH elongation and density. This question has been investigated in barley by comparing wild type and hairless mutant (*brb*) plants inoculated or not by different endomycorrhizal fungi. Mycorrhizas were found to substitute for RHs in P uptake, but the additional P was most often used less efficiently, in terms of plant growth, compared with P provided by RHs [55]. A similar study used several barley lines that were differentially affected in RH development (hairless, short or intermediate, or wild-type RH length phenotypes). The plants were naturally colonized by a live community present in the soil. Endomycorrhizal symbiosis did not fully compensate for the absence of RHs with regard to both P acquisition and biomass production [56]. A third series of similar experiments, using the hairless barley mutant *brb* and the corresponding wild-type genotype grown under well-watered or drought conditions showed that, with respect to biomass production, endomycorrhizal symbiosis compensated for the absence of RHs in the latter condition but not in the former [57]. Altogether, these reports indicate that AM associations can be less efficient in P-acquisition and biomass production compared with RHs in some environmental conditions.

Organic Compound Exudation, Plant Growth-Promoting Rhizobacteria, and Stimulation of RH Development

Plants exude large amounts of organic compounds into the soil, rendering the rhizosphere a rich niche for the development of microbial communities. The actual amount of carbon (C) invested in root exudation, which can vary from 5% to 50% of the net photosynthesized C [58,59], depends on the health of the plant, its rate of growth, its nutrient and water status, and its microbiota. Root exudation has been compared in wild type and hairless mutant barley plants, revealing that the amount of exuded C was three times higher in wild-type plants compared with the hairless mutant [60]. Furthermore, experiments carried out with wild type and hairless mutant barley plants showed that an absence of RHs significantly reduced the diversity of the bacterial community [61]. Bacterial attraction by root exudates probably involves selective chemotaxis processes [62].

The microbial community thriving in the rhizosphere can include up to 10^9 bacteria per gram of soil, belonging to diverse taxa [61,62]. Within this population, bacteria generically named plant growth promoting rhizobacteria (PGPR) can be recruited by roots to engage in beneficial interactions. PGPR promote plant growth via very diverse mechanisms, such as improved plant mineral nutrition resulting from solubilization of poorly soluble nutrient sources, production of phytohormones that affect root development, and protection against phytoparasites [63].

Promotion of RH development, likely to result in improved mineral nutrition, has been reported in response to diverse PGPR and in various plant species [64–71]. The increase in RH length can be important, by more than 100% [64,68,71], making this response to the bacterial inoculation the easiest to detect and, thus, the most straightforward way in laboratory experiments to check whether a given plant species can interact with a given PGPR.

Large differences in the capacity to promote RH elongation have been observed between PGPR strains [64]. This raises the question of whether such differences are correlated with PGPR capacity to promote plant growth and, thus, are indicative of symbiosis effectiveness. However, pathogenic strains of the bacterium *Pseudomonas syringae* have been shown to promote RH elongation in arabidopsis, similar to that seen with beneficial *Pseudomonas* spp. bacteria [72].

Plasticity of RH Development and Adaptation to External Conditions

RH development is strongly responsive to environmental factors, nutrient availability, and rhizosphere microbial communities (see earlier), as well as soil porosity, strength, and water content [73]. Different patterns of RH distribution within the root epidermis can be identified among plant species (Box 1) but the question of whether a given pattern has specific advantages and in what environmental conditions is poorly documented.

Studies in arabidopsis (RH distribution pattern 3; Box 1) to investigate how external biotic or abiotic conditions can impact epidermal cell differentiation and RH morphogenesis, have brought to light hormone-driven processes [74]. Evidence has been obtained that auxin and ethylene have major roles in the control of epidermal cell differentiation and RH development and that the pathways allowing these two hormones to affect RH formation are significantly congruent [54,74–77]. Proper auxin distribution is required for correct cell fate assignment and RH formation (both initiation site selection and tip growth). In arabidopsis, auxin regulates RH formation by acting downstream of RHD6, and probably primarily via RSL4 [78,79], two transcription factors with central roles in RH development (Box 2). Ethylene also acts on RH formation downstream of RHD6 [78]. Evidence is also available that jasmonic acid, strigolactones, and cytokinins are positive regulators of RH growth, whereas brassinosteroids and abscisic acid are negative regulators [54,74,75,80].

The widely reported increase in RH density and length in response to low P availability involves induction of RH-expressed auxin-inducible transcription factors, including RSL2 and RSL4 [81] and ethylene-mediated events with a role in the control of RH gene expression [82]. Promotion of RH development by PGPR species has also been shown to involve auxin [71], ethylene [65], and a complex interplay of auxin and ethylene signaling pathways [83]. Nevertheless, a case of PGPR-induced promotion of RH elongation poorly dependent on auxin and ethylene signaling mechanisms has been reported in arabidopsis [67].

Finally, although control of RH longevity is likely to be of major importance in root system adaptation to soil abiotic and biotic factors, RH longevity is still poorly documented. Reported values in barley vary from a few days to 2–3 weeks [84–86]. Evidence has been obtained that apoptosis-like programmed cell death (AL-PCD), characterized by protoplast retraction, nuclear DNA fragmentation, and sensitivity to inhibitors of caspase-3-like activity, occurs in arabidopsis RHs in response to heat shock, salt stress, and reactive oxygen species (ROS; H_2O_2) treatment [87,88]. Basal AL-PCD rates ranging from *ca.* 5% to 15% have been monitored in arabidopsis seedlings classically grown on agar plates [88]. Thus, it is tempting to hypothesize that the extension of functional RH zones might be regulated by AL-PCD in response to local environmental conditions.

Box 1. Root Hair Distribution Patterns

RH cell distribution within the root epidermis varies among angiosperms. The distribution patterns have been sorted into three basic types: random, alternating, and position-dependent, named types 1, 2, and 3, respectively [106,107] (Figure I). In type 1 development, displayed by, for example, *Medicago truncatula*, barley, and maize, RH cells can differentiate from any epidermal cell. This results in the absence of regular patterns, in contrast to types 2 and 3 development. Type 2, displayed by, for example, wheat, rice, and *Brachypodium*, involves asymmetry in the last cell division just before epidermal cells leave the meristematic zone, leading to the formation of two daughter cells that differ in size. Only the smaller cell differentiates into an RH cell. Thus, in each epidermal cell file along the root longitudinal axis, RH cells and non-hair cells alternate. In type 3 plants, such as *Arabidopsis thaliana* and Brassica, cell files comprising entirely RHs along the root longitudinal axis alternate with one or more non-hair cell files. Evidence has been obtained in Arabidopsis that this pattern results from position-dependent hair cell specification: RH cells are located over two underlying cortical cells (the H cell position), whereas non-hair cells are positioned over a single cortical cell (the N cell position; see Box 2 in the main text) [54]. However, this classification of RH distribution patterns into three major types do not describe the whole diversity regarding this trait since, for instance, a type 3 variant, in which long hairs differentiate from cells in H position and short hairs from cells in the N position, has been described [108].

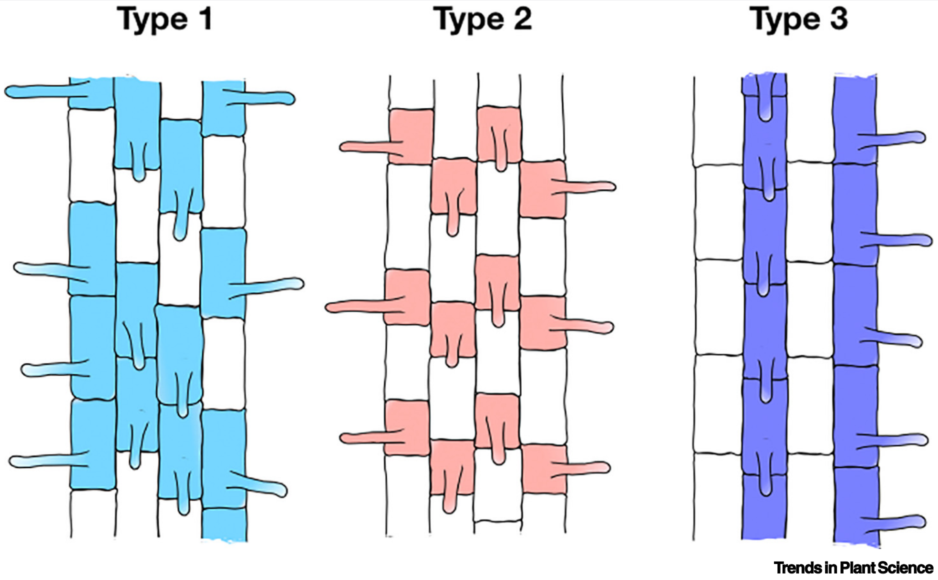

Figure I. Root Hair Distribution Patterns.

Quantitative Trait Loci of RH Production and Plant Yield

Quantitative trait loci (QTLs) of RH length have been identified in various crops (e.g., barley [89], maize [90], and wheat [10,91–93]). A seminal study of the correlation between RH length and biomass production and yield, reported by Gahoonia and Nielsen [19], explored the biological diversity within 38 barley cultivars. Large variations in RH length, from ~0.4 mm to >1.3 mm, were observed in hydroponically grown plants, consistent with variations thereafter observed in field conditions. Then, a set of ten representative cultivars was tested in field experiments, with different levels of soil P availability. The complete set of results indicated that barley genotypes with long RHs displayed higher tolerance to low P conditions, and expressed higher yield potentials both in low and high P soils [19]. In a similar experiment, characterization of barley mutant lines with various RH phenotypes, screened from a mutagenized population, showed that RH length was important for shoot P accumulation and biomass production, especially under combined water and phosphorus stress [94]. However, for grain yield, only the presence of RHs, and not RH length, was critical. The difference in RH length between the genotypes classified as ‘Short RH’ and ‘Long RH’ (0.54 mm vs 0.69 mm) in this report was small compared with the differences observed within the set of barley cultivars previously used by Gahoonia and Nielsen

Box 2. Arabidopsis Root Hair Development

Figure I describes the core of the current model of the regulation of RH development by the intrinsic developmental program in arabidopsis [54,75], which displays a type-3 RH distribution pattern (see Box 1 in the main text). The default fate for an epidermal cell is an RH cell, and entry in the non-hair cell developmental program involves expression of a transcription factor (TF) named GL2 (GLABRA2), which ultimately blocks the hair pathway. In a given epidermal cell, the level of GL2 expression, and thereby cell fate, is determined by the relative position of this cell with respect to the underlying cortical cells. An epidermal cell in contact with two cortical cells, a position which is named 'H' (for 'hair'), develops into an RH cell, whereas a cell in contact with a single cortical cell, a position named 'N' (for 'non hair'), enters the non-hair cell developmental program. Signals leading to this differentiation pattern are emitted by the cortical cell layer, and the signaling pathway involves JACKDAW (JKD), a zinc finger protein expressed in cortical cells, and SCRAMBLED (SCM), an RH plasmalemma receptor-like kinase. Perception of the signal together with a highly diversified and complex series of mechanisms contributes to tune the relative abundancy in N and H cells of two TFs, WER and CPC. Each of these TFs is able to form a complex with three other TFs, GL3, EGL3, and TTG1. In N cells, due to a larger abundancy of WER, a WER-GL3/EGL3-TTG1 complex is formed and activates the expression of GL2, which blocks the hair fate by inhibiting the expression of TFs, among which RHD6, required for RH formation. In H cells, CPC inhibits the function of the WER-GL3/EGL3-TTG1 complex by interfering with WER binding to GL3/EGL3 in a competitive manner, thereby leading to a CPC inactive complex with respect to GL2 expression. Absence of the block by GL2 allows expression of RHD6 and other TFs (RSL1 and RSL2) with major roles in RH initiation and elongation. The TF RSL4 is one of the direct targets of RHD6 and a major contributor to the expression of RH cell-specific genes involved in RH elongation.

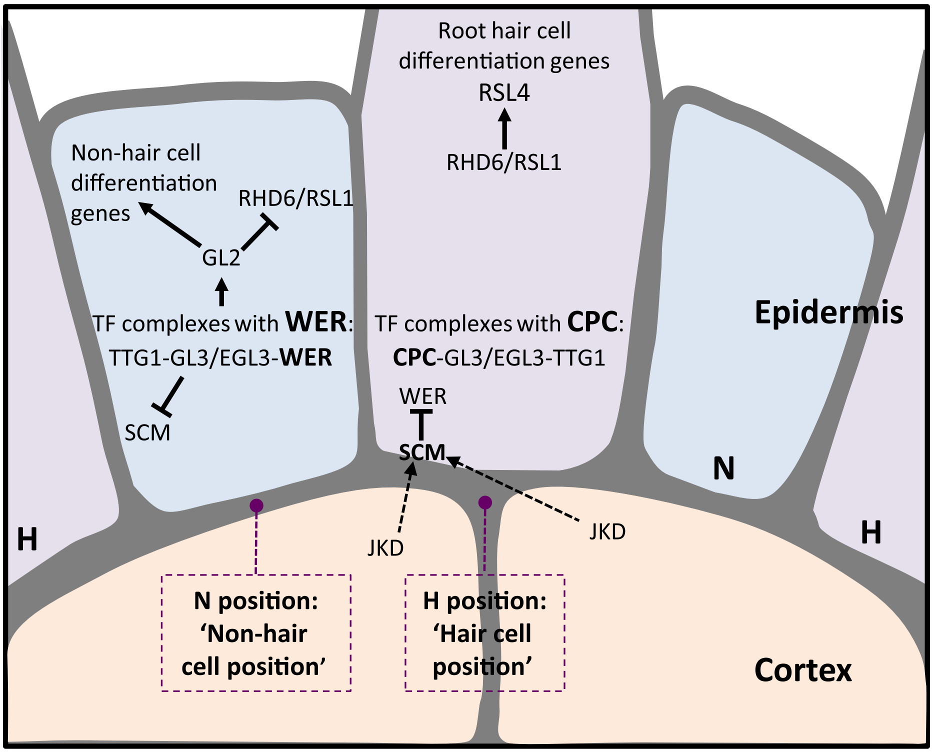

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Figure I. Arabidopsis Root Hair Development. Arrows indicate positive control. Blunted lines indicate negative regulation. Broken lines indicate intercellular signal movement.

[19]. Evidence that plant biomass production can be positively correlated to RH length in barley has also been provided by phenotyping 175 lines from a doubled-haploid (DH) population, using rhizosheath size as a surrogate for RH length and the normalized difference vegetation index (NDVI) as a surrogate for crop biomass. These analyses revealed colocation between QTLs for root rhizosheath size and NDVI-estimated biomass [89]. QTLs for RH length have

also been found to colocate with QTL for yield components in wheat [91]. However, further work is still required to investigate whether, and under what environmental conditions, longer RHs benefit grain yield.

Concluding Remarks and Future Perspectives

RHs have major roles in plant autotrophy and fitness by contributing to the anchorage of growing root tips into the soil, to soil mining for water and nutrient acquisition, and to interactions with soil microorganisms. The RH cell model is extensively used to decipher processes of cell fate and cell-type patterning in plants as well as the mechanisms involved in tip growth. However, it is clear that many questions regarding RH development and functions at the soil–root interface deserve to be further investigated, such as the mechanisms involved in epidermal cell fate in species displaying a RH patterning different from that in arabidopsis (Box 1), or the environmental and internal determinants of RH longevity and apoptosis-like programmed cell death.

In terms of crop breeding, evidence is available that the genetic variation in root system and RH traits can be used to reduce fertilization and irrigation inputs and increase crop yield in poor soil conditions [95,96]. With the objective of contributing to a vital new green revolution, strong efforts have been aimed at developing methodologies and imaging platforms for high-throughput phenotyping (HTP) of root trait variation in large genotype collections to identify promising germplasm and markers for selection. So far, the root traits that have been analyzed by such HTP approaches and shown to be positively associated with yield in field experiments, at least in some soil and environmental conditions, most often correspond to macroscopic features, such as ‘primary root length’ in oilseed rape [97], or ‘seminal root number’, ‘total root length’ [98], ‘narrow root angle’ (thought to result in a steep root phenotype) [99], or ‘root diameter’ [100] in wheat. None of the HTP methodologies used in these studies were reported to have included RH development in the targeted root traits, probably because the phenotyping procedure was not aimed at obtaining and analyzing high-resolution images. RH traits that have to be taken into account are length and density as well as the sensitivity of these parameters to abiotic and biotic conditions, such as reduced nutrient and water availability or presence of PGPR. It is also likely that the location and relative extension of the root zones bearing live RHs are important traits. HTP methodologies could also be used to screen, for instance, collections of crop cultivars together with collections of beneficial soil bacteria by phenotyping the RH responses to the inoculated bacteria before assessing the effects of selected bacterial partners on plant growth in soil conditions. Various low-cost HTP methodologies can be used/adapted for such research objectives, including the so-called ‘paper-roll’ and ‘pouch and wick’ setups and other 2D phenotyping methodologies [101]. Our group is currently developing a similar 2D HTP approach using rhizobox-like devices in which root growth occurs (Figure 1) on a piece of non-putrescible tissue under automatically controlled watering. More complex 3D phenotyping methodologies could also be developed using heterogeneous transparent substrates that mimic soil features [102,103].

RH phenotyping in plants grown in field conditions can provide valuable criteria for plant breeding programs. Gentle and careful washing of excavated root systems can allow quantification of RH traits, namely length and density [3,104], but this is poorly amenable to high-throughput procedures. By contrast, such analyses of RH genotypic variation in the field have provided information in line with the results of HTP analyses (paper-roll type) and have been found to be effective in breeding programs for edaphic stress tolerance in low-input agriculture [95,104]. Thus, evidence is already available that a combination of HTP approaches with assessment of the selected germplasm in field conditions can increase the efficiency and speed up plant breeding for low-input agriculture [95,96,105]. This suggests that further development/adaptation of HTP

Outstanding Questions

Q8

What are the sensing and signaling mechanisms allowing RH production and elongation to respond to soil features (nutrient ion availability, soil moisture, texture, porosity…)?

How long do RHs stay alive and functional in the soil and what are the internal and external determinants of RH lifespan?

Do the different RH distribution patterns have specific physiological advantages and under what environmental conditions?

What are the comparative cost–benefit ratios of RH production and mycorrhization, in terms of biomass production and grain yield?

What is the physiological meaning of the stimulation of RH elongation by rhizobacteria, and can this stimulation be operationally considered, in HTP methodologies, as an indication of an engagement in beneficial interactions?

Q9

What kinds of high-throughput root trait phenotyping methodologies could take into RHs consideration?

What have been the consequences of generations of selective breeding for increased yields in artificial soil conditions on the ability of plants to invest photosynthates in root development and functions?

methodologies enabling quantitative phenotyping of RH traits and of their responses to abiotic and biotic conditions would contribute significantly to such programs.

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Resources

[i] https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Ppatens

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