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## Looking for Root Hairs to Overcome Poor Soils

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1

## Q2 Review

## 3 Looking for Root Hairs to Overcome Poor Soils

Q4 Q3 Thanyakorn Rongsawat,<sup>1,2</sup> Jean-Benoît Peltier,<sup>1</sup> Jean-Christophe Boyer,<sup>1</sup> Anne-Aliénor Véry,<sup>1</sup>  
5 and Hervé Sentenac<sup>1,\*</sup>

6 **Breeding new cultivars allowing reduced fertilization and irrigation is a major chal-**  
7 **lenge. International efforts towards this goal focus on noninvasive methodologies,**  
8 **platforms for high-throughput phenotyping of large plant populations, and quanti-**  
9 **tative description of root traits as predictors of crop performance in environments**  
10 **with limited water and nutrient availability. However, these high-throughput analy-**  
11 **ses ignore one crucial component of the root system: root hairs (RHs). Here, we**  
12 **review current knowledge on RH functions, mainly in the context of plant**  
13 **hydromineral nutrition, and take stock of quantitative genetics data pointing at cor-**  
14 **relations between RH traits and plant biomass production and yield components.**

16 **Root Hairs Enlarge the Soil–Root Interface**

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

32 **Adhesion to Soil Particles, Soil Penetration, and Rhizosheath Formation**

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

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

## Highlights

Plant breeding for improved below-ground 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.

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## Key Figure

## Q1 Root System of Wheat Seedlings



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(See figure legend at the bottom of the next page.)

## 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.

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

### 81 Nutrient and Water Acquisition

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

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

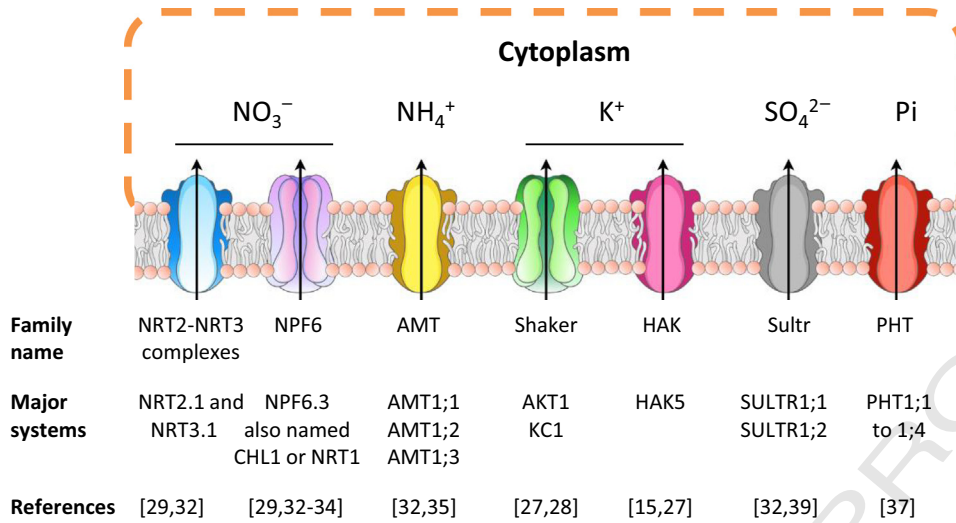
### 98 Ion Transport Systems at the RH Plasma Membrane

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

103  $\text{K}^+$  uptake from the soil by arabidopsis roots is essentially mediated by the high-affinity  $\text{K}^+$  trans-  
104 porter AtHAK5 and the **Shaker channels** AtAKT1 and AtKC1 [27]. Evidence is available that  
105 these three  $\text{K}^+$  transport systems are expressed in RHs [15,28].

106  $\text{NO}_3^-$  acquisition by roots involves transporters belonging to three different families: nitrate trans-  
107 porter 1/peptide transporter family (NPF), NRT2, and NRT3 (also named NAR2 for ‘nitrate assim-  
108 ilation related family’) [29]. The NPF family comprises the extensively studied AtNPF6.3  
109 ‘**transceptor**’ (endowed with a dual  $\text{NO}_3^-$  transport/signaling function [30]), initially named CHL1  
110 or AtNRT1.1. This membrane protein behaves both as a dual-affinity  $\text{NO}_3^-$  transporter and as a  
111  $\text{NO}_3^-$  sensor mediating  $\text{NO}_3^-$  regulated auxin transport, thereby having an important role in root de-  
112 velopment [29,31]. Transcriptome data provide evidence that *AtNPF6.3/AtNRT1.1* transcripts are  
113 present in arabidopsis RHs [32]. The NRT2 family comprises AtNRT2.1 and AtNRT2.2, which  
114 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.



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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].

118 heteromeric structures that contribute significantly to high-affinity  $\text{NO}_3^-$  uptake from the soil solution  
 119 [29,33]. *AtNRT2.1* and *AtNRT3.1* display expression in RHs as shown by reporter gene experiments  
 120 and/or transcriptome analyses [32,34]. RHs also express members of the AMT, PHT1, and Sultr  
 121 families, involved in ammonium [35], phosphate [36-38], and sulfate [39] uptake in arabidopsis,  
 122 respectively [32].

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

132 The sensitivity of RH length and density to nutrient and water availability in the soil (see earlier) may  
 133 involve a role of RHs as sensors of soil conditions. Such a hypothesis of early sensing of water  
 134 deficit conditions has received support from studies in barley wild type and hairless mutant plants.  
 135 Analysis of transcriptomes from roots that were sampled at the onset of a water stress revealed  
 136 that more genes were induced in the roots of the wild-type plants, including, for example, genes  
 137 involved in abscisic acid biosynthesis [45]. In terms of nutrient sensing, the arabidopsis  $\text{NO}_3^-$   
 138 transporter *AtNPF6.3/AtNRT1.1* and the Shaker  $\text{K}^+$  channel *AtAKT1*, which have both been  
 139 proposed to behave as transceptors, able to sense and signal the availability of their substrates,  
 140  $\text{NO}_3^-$  and  $\text{K}^+$ , respectively [30,46], are both expressed in RHs (see earlier).

### 141 **RH Production versus Engagement in Mycorrhizal Symbiosis**

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

151 Mycorrhizal fungal hyphae can have a smaller diameter (ca. 4  $\mu\text{m}$  for *Glomus intraradices* and  
152 5  $\mu\text{m}$  for *Glomus mosseae* [53]) compared with RHs (ca. 10  $\mu\text{m}$  in arabidopsis [54]), which  
153 allows exploration of smaller soil pores. Furthermore, they extend far beyond the limits of  
154 the RH cylinder. Thus, mycorrhizal symbiosis may be hypothesized to be more efficient for  
155 exploiting the soil compared with the promotion of RH elongation and density. This question  
156 has been investigated in barley by comparing wild type and hairless mutant (*brb*) plants  
157 inoculated or not by different endomycorrhizal fungi. Mycorrhizas were found to substitute  
158 for RHs in P uptake, but the additional P was most often used less efficiently, in terms of  
159 plant growth, compared with P provided by RHs [55]. A similar study used several barley  
160 lines that were differentially affected in RH development (hairless, short or intermediate, or  
161 wild-type RH length phenotypes). The plants were naturally colonized by a live community  
162 present in the soil. Endomycorrhizal symbiosis did not fully compensate for the absence of  
163 RHs with regard to both P acquisition and biomass production [56]. A third series of similar  
164 experiments, using the hairless barley mutant *brb* and the corresponding wild-type genotype  
165 grown under well-watered or drought conditions showed that, with respect to biomass  
166 production, endomycorrhizal symbiosis compensated for the absence of RHs in the latter  
167 condition but not in the former [57]. Altogether, these reports indicate that AM associations  
168 can be less efficient in P-acquisition and biomass production compared with RHs in some  
169 environmental conditions.

170

### 171 **Organic Compound Exudation, Plant Growth-Promoting Rhizobacteria, and** 172 **Stimulation of RH Development**

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

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

189 Promotion of RH development, likely to result in improved mineral nutrition, has been reported in  
Q5 response to diverse PGPR and in various plant species [64–71]. The increase in RH length can be  
191 important, by more than 100% [64,68,71], making this response to the bacterial inoculation the  
192 easiest to detect and, thus, the most straightforward way in laboratory experiments to check  
193 whether a given plant species can interact with a given PGPR.

194 Large differences in the capacity to promote RH elongation have been observed between PGPR  
195 strains [64]. This raises the question of whether such differences are correlated with PGPR  
196 capacity to promote plant growth and, thus, are indicative of symbiosis effectiveness. However,  
197 pathogenic strains of the bacterium *Pseudomonas syringae* have been shown to promote RH  
Q6 elongation in arabidopsis, similar to that seen with beneficial *Pseudomonas* spp. bacteria [72].

### 199 Plasticity of RH Development and Adaptation to External Conditions

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

205 Studies in arabidopsis (RH distribution pattern 3; Box 1) to investigate how external biotic or abi-  
206 otic conditions can impact epidermal cell differentiation and RH morphogenesis, have brought to  
207 light hormone-driven processes [74]. Evidence has been obtained that auxin and ethylene have  
208 major roles in the control of epidermal cell differentiation and RH development and that the path-  
209 ways allowing these two hormones to affect RH formation are significantly congruent [54,74–77].  
210 Proper auxin distribution is required for correct cell fate assignment and RH formation (both  
211 initiation site selection and tip growth). In arabidopsis, auxin regulates RH formation by acting  
212 downstream of RHD6, and probably primarily via RSL4 [78,79], two transcription factors with  
213 central roles in RH development (Box 2). Ethylene also acts on RH formation downstream of  
214 RHD6 [78]. Evidence is also available that jasmonic acid, strigolactones, and cytokinins are pos-  
215 itive regulators of RH growth, whereas brassinosteroids and abscisic acid are negative regulators  
216 [54,74,75,80].

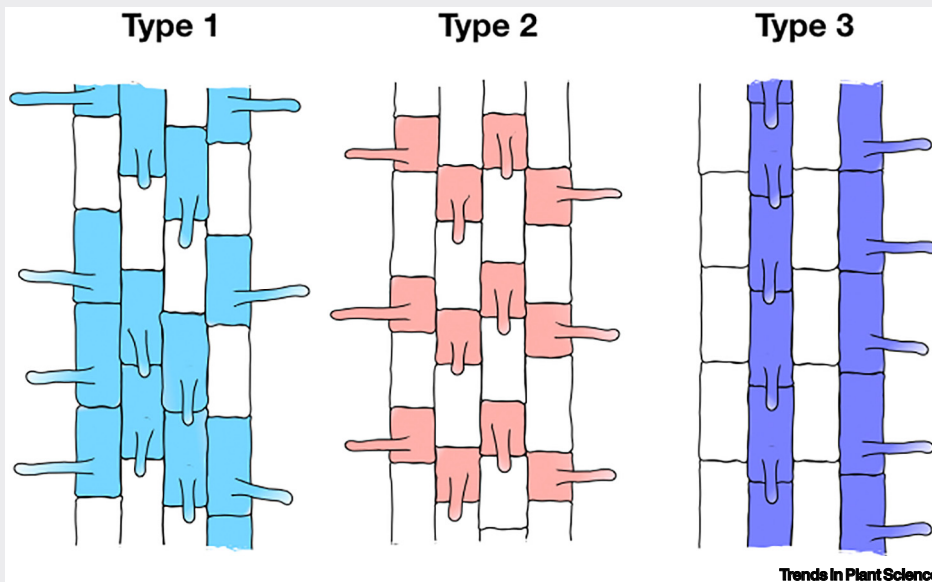
217 The widely reported increase in RH density and length in response to low P availability involves  
218 induction of RH-expressed auxin-inducible transcription factors, including RSL2 and RSL4 [81]  
219 and ethylene-mediated events with a role in the control of RH gene expression [82]. Promotion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

224 Finally, although control of RH longevity is likely to be of major importance in root system  
225 adaptation to soil abiotic and biotic factors, RH longevity is still poorly documented. Reported  
226 values in barley vary from a few days to 2–3 weeks [84–86]. Evidence has been obtained that  
227 apoptosis-like programmed cell death (AL-PCD), characterized by protoplast retraction, nuclear  
228 DNA fragmentation, and sensitivity to inhibitors of caspase-3-like activity, occurs in arabidopsis  
229 RHs in response to heat shock, salt stress, and reactive oxygen species (ROS; H<sub>2</sub>O<sub>2</sub>) treatment  
230 [87,88]. Basal AL-PCD rates ranging from ca. 5% to 15% have been monitored in arabidopsis  
231 seedlings classically grown on agar plates [88]. Thus, it is tempting to hypothesize that the  
232 extension of functional RH zones might be regulated by AL-PCD in response to local environmen-  
233 tal conditions.



b0.2 **Box 1. Root Hair Distribution Patterns**

b1.3 RH cell distribution within the root epidermis varies among angiosperms. The distribution patterns have been sorted into  
 b1.4 three basic types: random, alternating, and position-dependent, named types 1, 2, and 3, respectively [106,107] (Figure I).  
 b1.5 In type 1 development, displayed by, for example, *Medicago truncatula*, barley, and maize, RH cells can differentiate from  
 b1.6 any epidermal cell. This results in the absence of regular patterns, in contrast to types 2 and 3 development. Type 2,  
 b1.7 displayed by, for example, wheat, rice, and *Brachypodium*, involves asymmetry in the last cell division just before epidermal  
 b1.8 cells leave the meristematic zone, leading to the formation of two daughter cells that differ in size. Only the smaller cell  
 b1.9 differentiates into an RH cell. Thus, in each epidermal cell file along the root longitudinal axis, RH cells and non-hair cells  
 b1.10 alternate. In type 3 plants, such as *Arabidopsis thaliana* and Brassica, cell files comprising entirely RHs along the root  
 b1.11 longitudinal axis alternate with one or more non-hair cell files. Evidence has been obtained in *Arabidopsis* that this pattern  
 b1.12 results from position-dependent hair cell specification: RH cells are located over two underlying cortical cells (the H cell  
 b1.13 position), whereas non-hair cells are positioned over a single cortical cell (the N cell position; see Box 2 in the main text)  
 b1.14 [54]. However, this classification of RH distribution patterns into three major types do not describe the whole diversity  
 b1.15 regarding this trait since, for instance, a type 3 variant, in which long hairs differentiate from cells in H position and short  
 b1.16 hairs from cells in the N position, has been described [108].



b1.20 **Figure I. Root Hair Distribution Patterns.**

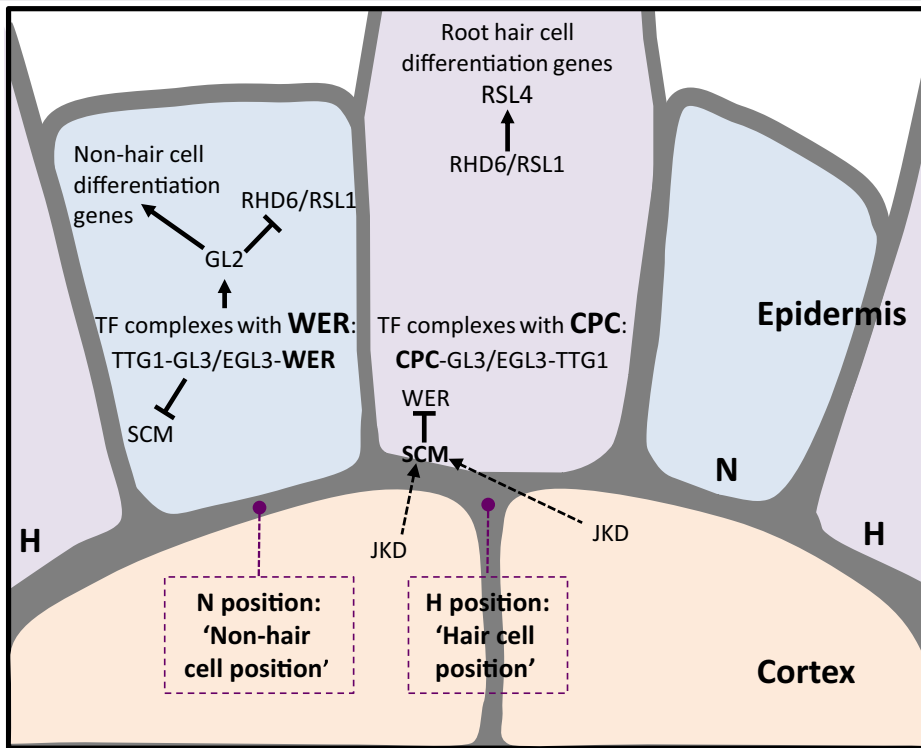
b1.18

**Q7 Quantitative Trait Loci of RH Production and Plant Yield**

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

**Box 2. Arabidopsis Root Hair Development**

Figure 1 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 abundance 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 abundance 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 1. 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

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

258

### 259 Concluding Remarks and Future Perspectives

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

268 In terms of crop breeding, evidence is available that the genetic variation in root system and RH  
 269 traits can be used to reduce fertilization and irrigation inputs and increase crop yield in poor soil  
 270 conditions [95,96]. With the objective of contributing to a vital new green revolution, strong efforts  
 271 have been aimed at developing methodologies and imaging platforms for high-throughput  
 272 phenotyping (HTP) of root trait variation in large genotype collections to identify promising  
 273 germplasm and markers for selection. So far, the root traits that have been analyzed by such  
 274 HTP approaches and shown to be positively associated with yield in field experiments, at least  
 275 in some soil and environmental conditions, most often correspond to macroscopic features,  
 276 such as ‘primary root length’ in oilseed rape [97], or ‘seminal root number’, ‘total root length’  
 277 [98], ‘narrow root angle’ (thought to result in a steep root phenotype) [99], or ‘root diameter’  
 278 [100] in wheat. None of the HTP methodologies used in these studies were reported to have in-  
 279 cluded RH development in the targeted root traits, probably because the phenotyping procedure  
 280 was not aimed at obtaining and analyzing high-resolution images. RH traits that have to be taken  
 281 into account are length and density as well as the sensitivity of these parameters to abiotic and  
 282 biotic conditions, such as reduced nutrient and water availability or presence of PGPR. It is  
 283 also likely that the location and relative extension of the root zones bearing live RHs are important  
 284 traits. HTP methodologies could also be used to screen, for instance, collections of crop cultivars  
 285 together with collections of beneficial soil bacteria by phenotyping the RH responses to the  
 286 inoculated bacteria before assessing the effects of selected bacterial partners on plant growth  
 287 in soil conditions. Various low-cost HTP methodologies can be used/adapted for such research  
 288 objectives, including the so-called ‘paper-roll’ and ‘pouch and wick’ setups and other 2D  
 289 phenotyping methodologies [101]. Our group is currently developing a similar 2D HTP approach  
 290 using rhizobox-like devices in which root growth occurs (Figure 1) on a piece of non-putrescible  
 291 tissue under automatically controlled watering. More complex 3D phenotyping methodologies  
 292 could also be developed using heterogeneous transparent substrates that mimic soil features  
 293 [102,103].

294 RH phenotyping in plants grown in field conditions can provide valuable criteria for plant breeding  
 295 programs. Gentle and careful washing of excavated root systems can allow quantification of RH  
 296 traits, namely length and density [3,104], but this is poorly amenable to high-throughput proce-  
 297 dures. By contrast, such analyses of RH genotypic variation in the field have provided information  
 298 in line with the results of HTP analyses (paper-roll type) and have been found to be effective in  
 299 breeding programs for edaphic stress tolerance in low-input agriculture [95,104]. Thus, evidence  
 300 is already available that a combination of HTP approaches with assessment of the selected  
 301 germplasm in field conditions can increase the efficiency and speed up plant breeding for low-  
 302 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?

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

Q9

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?

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

305

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310 **Resources**

311 <sup>1</sup> [https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org\\_Ppatens](https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Ppatens)

312

313 **References**

- 316 1. Lynch, J.P. (2019) Root phenotypes for improved nutrient  
317 capture: an underexploited opportunity for global agriculture.  
318 *New Phytol.* 223, 548–564
- 319 2. Zhu, Y.H. *et al.* (2019) Evolutionary agroecology: trends in root  
320 architecture during wheat breeding. *Evol. Appl.* 12, 733–743
- 321 3. Dittmer, H.J. (1937) A quantitative study of the roots and root  
322 hairs of a winter rye plant (*Secale cereale*). *Am. J. Bot.* 24,  
323 417–420
- 324 4. Choi, H.-S. *et al.* (2019) Root hairs enhance *Arabidopsis* seed-  
325 ling survival upon soil disruption. *Sci. Rep.* 9, 11181
- 326 5. Bengough, A.G. *et al.* (2016) Root hairs aid soil penetration by  
327 anchoring the root surface to pore walls. *J. Exp. Bot.* 67,  
328 1071–1078
- 329 6. Galloway, A.F. *et al.* (2020) Cereal root exudates contain highly  
330 structurally complex polysaccharides with soil-binding properties.  
331 *Plant J.* 103, 1666–1678
- 332 7. Huang, Y. *et al.* (2016) Nanospherical arabinogalactan proteins  
333 are a key component of the high-strength adhesive secreted  
334 by English ivy. *Proc. Natl. Acad. Sci. U. S. A.* 113, E3193–E3202
- 335 8. Bailey, P.H. *et al.* (2002) The role of root system architecture  
336 and root hairs in promoting anchorage against uprooting  
337 forces in *Allium cepa* and root mutants of *Arabidopsis thaliana*.  
338 *J. Exp. Bot.* 53, 333–340
- 339 9. Pang, J. *et al.* (2017) Unwrapping the rhizosphere. *Plant Soil*  
340 418, 129–139
- 341 10. Delhaize, E. *et al.* (2015) The genetics of rhizosphere size in a  
342 multiparent mapping population of wheat. *J. Exp. Bot.* 66,  
343 4527–4536
- 344 11. George, T.S. *et al.* (2014) Understanding the genetic control  
345 and physiological traits associated with rhizosphere production  
346 by barley (*Hordeum vulgare*). *New Phytol.* 203, 195–205
- 347 12. Brown, L.K. *et al.* (2017) The rhizosphere – a potential trait for  
348 future agricultural sustainability occurs in orders throughout  
349 the angiosperms. *Plant Soil* 418, 115–128
- 350 13. Bates, T.R. and Lynch, J.P. (1996) Stimulation of root hair elongation  
351 in *Arabidopsis thaliana* by low phosphorus availability.  
352 *Plant Cell Environ.* 19, 529–538
- 353 14. Janes, G. *et al.* (2018) Cellular patterning of *Arabidopsis* roots  
354 under low phosphate conditions. *Front. Plant Sci.* 9, 735
- 355 15. Ahn, S.J. *et al.* (2004) Expression of KT/KUP genes in  
356 *Arabidopsis* and the role of root hairs in K<sup>+</sup> uptake. *Plant Physiol.*  
357 134, 1135–1145
- 358 16. Canales, J. *et al.* (2017) Nitrate induction of root hair density is  
359 mediated by TGA1/TGA4 and CPC transcription factors in  
360 *Arabidopsis thaliana*. *Plant J.* 92, 305–316
- 361 17. Haling, R.E. *et al.* (2013) Root hairs improve root penetration,  
362 root-soil contact, and phosphorus acquisition in soils of different  
363 strength. *J. Exp. Bot.* 64, 3711–3721
- 364 18. Tanaka, N. *et al.* (2014) Characteristics of a root hair-less line of  
365 *Arabidopsis thaliana* under physiological stresses. *J. Exp. Bot.*  
366 65, 1497–1512
- 367 19. Gahoonia, T.S. and Nielsen, N.E. (2004) Barley genotypes with  
368 long root hairs sustain high grain yields in low-P field. *Plant Soil*  
369 262, 55–62
- 370 20. Klinsawang, S. (2018) Effects of root hair length on potassium  
371 acquisition in rice (*Oryza sativa* L.). *Appl. Ecol. Environ. Res.* 16,  
372 1609–1620
21. Babourina, O. *et al.* (2001) K<sup>+</sup> transport by *Arabidopsis* root  
373 hairs at low pH. *Funct. Plant Biol.* 28, 637
- 374 22. Lew, R.R. (1998) Immediate and steady state extracellular  
375 ionic fluxes of growing *Arabidopsis thaliana* root hairs under  
376 hyperosmotic and hypoosmotic conditions. *Physiol. Plant.*  
377 104, 397–404
- 378 23. Meharg, A.A. and Blatt, M.R. (1995) NO<sub>3</sub><sup>-</sup> transport across the  
379 plasma membrane of *Arabidopsis thaliana* root hairs: kinetic  
380 control by pH and membrane voltage. *J. Membr. Biol.* 145,  
381 49–66
- 382 24. Gahoonia, T.S. and Nielsen, N.E. (1998) Direct evidence on  
383 participation of root hairs in phosphorus (<sup>32</sup>P) uptake from  
384 soil. *Plant Soil* 198, 147–152
- 385 25. Ahmed, M.A. *et al.* (2018) Hydraulic processes in roots and the  
386 rhizosphere pertinent to increasing yield of water-limited grain  
387 crops: a critical review. *J. Exp. Bot.* 69, 3255–3265
- 388 26. Carminati, A. *et al.* (2017) Root hairs enable high transpiration  
389 rates in drying soils. *New Phytol.* 216, 771–781
- 390 27. Véry, A.A. *et al.* (2014) Molecular biology of K<sup>+</sup> transport across  
391 the plant cell membrane: what do we learn from comparison  
392 of be6 ween plant species. *J. Plant Physiol.* 171, 748–769
- 393 28. Reintanz, B. *et al.* (2002) AtKC1, a silent *Arabidopsis* potas-  
394 sium channel alpha -subunit modulates root hair K<sup>+</sup> influx.  
395 *Proc. Natl. Acad. Sci. U. S. A.* 99, 4079–4084
- 396 29. Wang, Y.Y. *et al.* (2018) Nitrate transport, signaling, and use  
397 efficiency. *Annu. Rev. Plant Biol.* 69, 85–122
- 398 30. Gojon, A. *et al.* (2011) Nitrate transporter(s) in plants. *J. Exp.*  
399 *Bot.* 62, 2299–2308
- 400 31. Krouk, G. *et al.* (2010) Nitrate-regulated auxin transport by  
401 NRT1.1 defines a mechanism for nutrient sensing in plants.  
402 *Dev. Cell* 18, 927–937
- 403 32. Huang, L. *et al.* (2017) Diversification of root hair development  
404 genes in vascular plants. *Plant Physiol.* 174, 1697–1712
- 405 33. Yong, Z. *et al.* (2010) Characterization of an intact two-  
406 component high-affinity nitrate transporter from *Arabidopsis*  
407 roots. *Plant J.* 63, 739–748
- 408 34. Nazoa, P. *et al.* (2003) Regulation of the nitrate transporter  
409 gene AtNRT2.1 in *Arabidopsis thaliana*: responses to nitrate,  
410 amino acids and developmental stage. *Plant Mol. Biol.* 52,  
411 689–703
- 412 35. Yuan, L. *et al.* (2007) The organization of high-affinity ammonium  
413 uptake in *Arabidopsis* roots depends on the spatial arrangement  
414 and biochemical properties of AMT1-type transporters. *Plant Cell*  
415 19, 2636–2652
- 416 36. Młodzińska, E. and Zbońska, M. (2016) Phosphate uptake and  
417 allocation - a closer look at *Arabidopsis thaliana* L. and *Oryza*  
418 *sativa* L. *Front. Plant Sci.* 7, 1198
- 419 37. Nussaume, L. *et al.* (2011) Phosphate import in plants: focus  
420 on the PHT1 transporters. *Front. Plant Sci.* 2, 83
- 421 38. Wang, F. *et al.* (2018) Molecular mechanisms of phosphate  
422 transport and signaling in higher plants. *Semin. Cell Dev. Biol.*  
423 74, 114–122
- 424 39. Takahashi, H. (2019) Sulfate transport systems in plants:  
425 functional diversity and molecular mechanisms underlying reg-  
426 ulatory coordination. *J. Exp. Bot.* 70, 4075–4087
- 427 40. Damiani, I. *et al.* (2016) Nod factor effects on root hair-specific  
428 transcriptome of *Medicago truncatula*: focus on plasma  
429

- 430 membrane transport systems and reactive oxygen species  
431 networks. *Front. Plant Sci.* 7, 794
- 432 41. De Michele, R. *et al.* (2012) Ammonium and urea transporter  
433 inventory of the *Selaginella* and *Physcomitrella* genomes.  
434 *Front. Plant Sci.* 3, 62
- 435 42. Garcíadeblás, B. *et al.* (2007) Potassium transport systems in  
436 the moss *Physcomitrella patens*: pphak1 plants reveal the  
437 complexity of potassium uptake. *Plant J.* 52, 1080–1093
- 438 43. Takahashi, H. *et al.* (2012) Evolutionary relationships and func-  
439 tional diversity of plant sulfate transporters. *Front. Plant Sci.* 2,  
440 119–128
- 441 44. Tsujimoto, R. *et al.* (2007) Distinct roles of nitrate and nitrite in  
442 regulation of expression of the nitrate transport genes in the  
443 moss *Physcomitrella patens*. *Plant Cell Physiol.* 48, 484–497
- 444 45. Kwasniewski, M. *et al.* (2016) Transcriptome analysis reveals  
445 the role of the root hairs as environmental sensors to maintain  
446 plant functions under water-deficiency conditions. *J. Exp. Bot.*  
447 67, 1079–1094
- 448 46. Shahzad, Z. and Amtmann, A. (2017) Food for thought: how  
449 nutrients regulate root system architecture. *Curr. Opin. Plant  
450 Biol.* 39, 80–87
- 451 47. Orfanoudakis, M. *et al.* (2010) Both the arbuscular mycorrhizal  
452 fungus *Gigaspora rosea* and *Frankia* increase root system  
453 branching and reduce root hair frequency in *Alnus glutinosa*.  
454 *Mycorrhiza* 20, 117–126
- 455 48. Sun, X.-G. and Tang, M. (2013) Effect of arbuscular mycorrhizal  
456 fungi inoculation on root traits and root volatile organic com-  
457 pound emissions of *Sorghum bicolor*. *S. Afr. J. Bot.* 88,  
458 373–379
- 459 49. Wu, Q.S. *et al.* (2016) Mycorrhiza alters the profile of root hairs  
460 in trifoliolate orange. *Mycorrhiza* 26, 237–247
- 461 50. Liu, C.Y. *et al.* (2018) Mycorrhiza stimulates root-hair growth  
462 and IAA synthesis and transport in trifoliolate orange under  
463 drought stress. *Sci. Rep.* 8, 1978
- 464 51. Ditegou, F.A. *et al.* (2000) Root hair elongation is inhibited by  
465 hypaphorine, the indole alkaloid from the ectomycorrhizal fun-  
466 gus *Pisolithus tinctorius*, and restored by indole-3-acetic acid.  
467 *Planta* 211, 722–728
- 468 52. Rigas, S. *et al.* (2013) Root gravitropism and root hair develop-  
469 ment constitute coupled developmental responses regulated by  
470 auxin homeostasis in the *Arabidopsis* root apex. *New  
471 Phytol.* 197, 1130–1141
- 472 53. Drew, E.A. *et al.* (2003) Beyond the rhizosphere: growth and  
473 function of arbuscular mycorrhizal external hyphae in sands of  
474 varying pore sizes. *Plant Soil* 251, 105–114
- 475 54. Grierson, C. *et al.* (2014) Root hairs. *Arabidopsis Book* 12,  
476 e0172
- 477 55. Jakobsen, I. *et al.* (2005) Contrasting phosphate acquisition of  
478 mycorrhizal fungi with that of root hairs using the root hairless  
479 barley mutant. *Plant Cell Environ.* 28, 928–938
- 480 56. Brown, L.K. *et al.* (2013) Interactions between root hair length  
481 and arbuscular mycorrhizal colonisation in phosphorus defi-  
482 cient barley (*Hordeum vulgare*). *Plant Soil* 372, 195–205
- 483 57. Li, T. *et al.* (2014) Relative importance of an arbuscular mycor-  
484 rhizal fungus (*Rhizophagus intraradices*) and root hairs in plant  
485 drought tolerance. *Mycorrhiza* 24, 595–602
- 486 58. Haichar, F.E.Z. *et al.* (2016) Stable isotope probing of carbon  
487 flow in the plant holobiont. *Curr. Opin. Biotechnol.* 41, 9–13
- 488 59. Venturi, V. and Keel, C. (2016) Signaling in the rhizosphere.  
489 *Trends Plant Sci.* 21, 187–198
- 490 60. Holz, M. *et al.* (2018) Root hairs increase rhizosphere extension  
491 and carbon input to soil. *Ann. Bot.* 121, 61–69
- 492 61. Robertson-Albertyn, S. *et al.* (2017) Root hair mutations  
493 displace the barley rhizosphere microbiota. *Front. Plant Sci.*  
494 8, 1094
- 495 62. Bulgarelli, D. *et al.* (2013) Structure and functions of the bacte-  
496 rial microbiota of plants. *Annu. Rev. Plant Biol.* 64, 807–838
- 497 63. Vacheron, J. *et al.* (2013) Plant growth-promoting rhizobacteria  
498 and root system functioning. *Front. Plant Sci.* 4, 356
- 499 64. Contesto, C. *et al.* (2008) Effects of rhizobacterial ACC deami-  
500 nase activity on *Arabidopsis* indicate that ethylene mediates  
501 local root responses to plant growth-promoting rhizobacteria.  
502 *Plant Sci.* 175, 178–189
- 503 65. Galland, M. *et al.* (2012) The ethylene pathway contributes  
504 to root hair elongation induced by the beneficial bacteria  
*Phyllobacterium brassicacearum* STM196. *Plant Sci.* 190,  
74–81
66. Jain, D.K. and Patriquin, D.G. (1984) Root hair deformation,  
bacterial attachment, and plant growth in wheat-*Azospirillum*  
associations. *Appl. Environ. Microbiol.* 48, 1208–1213
67. López-Bucio, J. *et al.* (2007) *Bacillus megaterium* rhizobacteria  
promote growth and alter root-system architecture through an  
auxin- and ethylene-independent signaling mechanism in  
*Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 20, 207–217
68. Poitout, A. *et al.* (2017) Local signalling pathways regulate the  
*Arabidopsis* root developmental response to *Mesorhizobium*  
*loti* inoculation. *J. Exp. Bot.* 68, 1199–1211
69. Ribaud, C.M. *et al.* (2006) *Azospirillum* sp. promotes root hair  
development in tomato plants through a mechanism that  
involves ethylene. *J. Plant Growth Regul.* 25, 175–185
70. Vacheron, J. *et al.* (2018) Differential contribution of plant-  
beneficial functions from *Pseudomonas kilonensis* F113  
to root system architecture alterations in *Arabidopsis*  
*thaliana* and *Zea mays*. *Mol. Plant-Microbe Interact.* 31,  
212–223
71. Zamioudis, C. *et al.* (2013) Unraveling root developmental pro-  
grams initiated by beneficial *Pseudomonas* spp. bacteria. *Plant  
Physiol.* 162, 304–318
72. Pecenková, T. *et al.* (2017) Early *Arabidopsis* root hair growth  
stimulation by pathogenic strains of *Pseudomonas syringae*.  
*Ann. Bot.* 120, 437–446
73. Haling, R.E. *et al.* (2014) Root hair length and rhizosphere mass  
depend on soil porosity, strength and water content in barley  
genotypes. *Planta* 239, 643–651
74. Vissenberg, K. *et al.* (2020) Hormonal regulation of root hair  
growth and responses to the environment in *Arabidopsis*.  
*J. Exp. Bot.* 71, 2412–2427
75. Cui, S. *et al.* (2018) Regulation and functional diversification of  
root hairs. *Semin. Cell Dev. Biol.* 83, 115–122
76. Bruex, A. *et al.* (2012) A gene regulatory network for root  
epidermis cell differentiation in *Arabidopsis*. *PLoS Genet.* 8,  
e1002446
77. Zhang, S. *et al.* (2016) Multiple phytohormones promote root  
hair elongation by regulating a similar set of genes in the root  
epidermis in *Arabidopsis*. *J. Exp. Bot.* 67, 6363–6372
78. Masucci, J.D. and Schiefelbein, J.W. (1996) Hormones act  
downstream of TTG and GL2 to promote root hair outgrowth  
during epidermis development in the *Arabidopsis* root. *Plant  
Cell* 8, 1505–1517
79. Yi, K. *et al.* (2010) A basic helix-loop-helix transcription factor  
controls cell growth and size in root hairs. *Nat. Genet.* 42,  
264–267
80. Han, G. *et al.* (2020) *Arabidopsis* ZINC FINGER PROTEIN1  
acts downstream of GL2 to repress root hair initiation and  
elongation by directly suppressing bHLH genes. *Plant Cell*  
32, 206–225
81. Bhosale, R. *et al.* (2018) A mechanistic framework for auxin  
dependent *Arabidopsis* root hair elongation to low external  
phosphate. *Nat. Commun.* 9, 1409
82. Song, L. *et al.* (2016) The molecular mechanism of ethylene-  
mediated root hair development induced by phosphate  
starvation. *PLoS Genet.* 12, e1006194
83. Poupin, M.J. *et al.* (2016) A complex molecular interplay  
of auxin and ethylene signaling pathways is involved in  
*Arabidopsis* growth promotion by *Burkholderia phytofirmans*  
PsJN. *Front. Plant Sci.* 7, 492
84. Henry, C.M. and Deacon, J.W. (1981) Natural (non-pathogenic)  
death of the cortex of wheat and barley seminal roots, as evi-  
denced by nuclear staining with acridine orange. *Plant Soil* 60,  
255–274
85. Holden, J. (1975) Use of nuclear staining to assess rates of  
cell death in cortices of cereal roots. *Soil Biol. Biochem.* 7,  
333–334
86. McElgunn, J.D. and Harrison, C.M. (1969) Formation, elongation,  
and longevity of barley root hairs. *Agron. J.* 61, 79–81
87. Hogg, B.V. *et al.* (2011) An *in vivo* root hair assay for determi-  
ning rates of apoptotic-like programmed cell death in plants.  
*Plant Methods* 7, 45
88. Tan, K. *et al.* (2016) Nuclear dynamics and programmed cell  
death in *Arabidopsis* root hairs. *Plant Sci.* 253, 77–85

580	89.	Gong, X. and McDonald, G. (2017) QTL mapping of root traits in phosphorus-deficient soils reveals important genomic regions for improving NDVI and grain yield in barley. <i>Theor. Appl. Genet.</i> 130, 1885–1902	611
581			612
582			613
583	90.	Zhu, J. <i>et al.</i> (2005) Mapping of QTL controlling root hair length in maize ( <i>Zea mays</i> L.) under phosphorus deficiency. <i>Plant Soil</i> 270, 299–310	614
584			615
585			616
586			617
587	91.	Horn, R. <i>et al.</i> (2016) Mapping of quantitative trait loci for root hair length in wheat identifies loci that co-locate with loci for yield components. <i>J. Exp. Bot.</i> 67, 4535–4543	618
588			619
589			620
590	92.	James, R.A. <i>et al.</i> (2016) Rhizosheaths on wheat grown in acid soils: phosphorus acquisition efficiency and genetic control. <i>J. Exp. Bot.</i> 67, 3709–3718	621
591			622
592			623
593	93.	Liu, M. <i>et al.</i> (2017) Analysis of aneuploid lines of bread wheat to map chromosomal locations of genes controlling root hair length. <i>Ann. Bot.</i> 119, 1333–1341	624
594			625
595			626
596	94.	Brown, L.K. <i>et al.</i> (2012) What are the implications of variation in root hair length on tolerance to phosphorus deficiency in combination with water stress in barley ( <i>Hordeum vulgare</i> ). <i>Ann. Bot.</i> 110, 319–328	627
597			628
598			629
599			630
600	95.	Burridge, J.D. <i>et al.</i> (2019) A case study on the efficacy of root phenotypic selection for edaphic stress tolerance in low-input agriculture: common bean breeding in Mozambique. <i>Field Crops Res.</i> 244, 107612	631
601			632
602			633
603			634
604	96.	Strock, C.F. <i>et al.</i> (2019) Seedling root architecture and its relationship with seed yield across diverse environments in <i>Phaseolus vulgaris</i> . <i>Field Crop Res.</i> 237, 53–64	635
605			636
606			637
607	97.	Thomas, C.L. <i>et al.</i> (2016) High-throughput phenotyping (HTP) identifies seedling root traits linked to variation in seed yield and nutrient capture in field-grown oilseed rape ( <i>Brassica napus</i> L.). <i>Ann. Bot.</i> 118, 655–665	638
608			639
609			640
610			641
	98.	Xie, Q. <i>et al.</i> (2017) Identifying seedling root architectural traits associated with yield and yield components in wheat. <i>Ann. Bot.</i> 119, 1115–1129	611
			612
			613
	99.	Khokhar, J.S. <i>et al.</i> (2019) Juvenile root traits show limited correlation with grain yield, yield components and grain mineral composition traits in Indian wheat under hostile soils. <i>Cereal Res. Commun.</i> 47, 362–382	614
			615
			616
			617
	100.	Bai, C. <i>et al.</i> (2019) The relationships between seedling root screens, root growth in the field and grain yield for wheat. <i>Plant Soil</i> 440, 311–326	618
			619
			620
	101.	Downie, H.F. <i>et al.</i> (2015) Challenges and opportunities for quantifying roots and rhizosphere interactions through imaging and image analysis. <i>Plant Cell Environ.</i> 38, 1213–1232	621
			622
	102.	Downie, H.F. <i>et al.</i> (2012) Transparent soil for imaging the rhizosphere. <i>PLoS ONE</i> 7, e44276	623
			624
			625
	103.	Ma, L. <i>et al.</i> (2019) Hydrogel-based transparent soils for root phenotyping <i>in vivo</i> . <i>Proc. Natl. Acad. Sci. U. S. A.</i> 116, 11063–11068	626
			627
			628
	104.	Miguel, M.A. (2015) Pene synergism between root hair length and basal root growth angle for phosphorus acquisition. <i>Plant Physiol.</i> 167, 1430–1439	629
			630
	105.	White, P.J. (2019) Root traits benefitting crop production in environments with limited water and nutrient availability. <i>Ann. Bot.</i> 124, 883–890	631
			632
			633
			634
	106.	Clowes, F.A.L. (2000) Pattern in root meristem development in angiosperms. <i>New Phytol.</i> 146, 83–94	635
			636
	107.	Marzec, M. <i>et al.</i> (2014) The evolutionary context of root epidermis cell patterning in grasses (Poaceae). <i>Plant Signal. Behav.</i> 9, e27972	637
			638
	108.	Tsai, S.L. <i>et al.</i> (2003) The root epidermis of <i>Echium plantagineum</i> L.: a novel type of pattern based on the distribution of short and long root hairs. <i>Planta</i> 217, 238–244	639
			640
			641