Root Hair Development in the Grasses: What We Already Know and What We Still Need to Know[1]

Marek Marzec*, Michael Melzer, and Iwona Szarejko

Department of Genetics, Faculty of Biology and Environmental Protection, University of Silesia, 40–032 Katowice, Poland (M.Ma., I.S.); and Department of Physiology and Cell Biology, Leibniz Institute of Plant Genetics and Crop Plant Research, D–06466 Gatersleben, Germany (M.Me.)

ORCID IDs: 0000-0002-5261-6662 (M.Ma.); 0000-0001-7390-7234 (I.S.).

A priority in many crop improvement programs for a long time has been to enhance the tolerance level of plants to both abiotic and biotic stress. Recognition that the root system is the prime determinant of a plant's ability to extract both water and minerals from the soil implies that its architecture is an important variable underlying a cultivar's adaptation. The density and/or length of the root hairs (RHs) that are formed are thought to have a major bearing on the plant's performance under stressful conditions. Any attempt to improve a crop's root system will require a detailed understanding of the processes of RH differentiation. Recent progress in uncovering the molecular basis of root epidermis specialization has been recorded in the grasses. This review seeks to present the current view of RH differentiation in grass species. It combines what has been learned from molecular-based analyses, histological studies, and observation of the phenotypes of both laboratory- and field-grown plants.

WHY ARE INVESTIGATIONS OF ROOT HAIR DEVELOPMENT IMPORTANT?

The root epidermis houses two distinct cell types: trichoblasts, which produce tubular outgrowths that are called root hairs (RHs), and atrichoblasts, which do not. It has been postulated for about a century that RHs are important for plant growth and development, since they are intimately involved in the uptake of both soil water and mineral nutrients, in the anchoring of the root as it penetrates the soil, and in interactions with the soil microfauna (Comber, 1922). The root system of an individual cereal rye (*Secale cereale*) plant develops up to 14 billion RHs, thereby increasing its root surface area by some 400 m^2 (Dittmer, 1937). This large increase in the extent of the contact between the root system and the soil is postulated to have a major impact on the plant's capacity to take up both water and minerals (Richardson et al., 2011; Brown et al., 2012; Haling et al., 2014). A number of indications have emerged that the RHs are important for the ability of grass species to maintain a sufficient level of water and nutrient acquisition when challenged by drought and/or a nutrient deficiency. The participation of the RHs in the uptake of phosphorus (P) has been identified in barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), maize (*Zea mays*), and bermudagrass (*Cynodon dactylon*; Green et al., 1991; Gahoonia et al., 1997; Brown et al., 2013b). In barley (Brown et al., 2012) and maize (Zhu et al., 2010), low P availability induces an increase in RH length, which is assumed to boost the efficiency of P uptake (Haling et al., 2013). Under P-limiting conditions, up to 90% of the mineral appears to be taken up by the RHs in different plant species (Fohse et al., 1991), a proportion that readily explains the major difference in the P acquisition capacity between barley accessions that form short as opposed to long RHs (Gahoonia and Nielsen, 2004; Brown et al., 2012). The importance of RHs for both cadmium and zinc acquisition has been illustrated by comparing the performance of the barley root-hairless mutant *bald root barley* (*brb*) with that of its parent cv Pallas. When wild-type and mutant plants are provided with sufficient zinc, both shoot dry matter and shoot zinc content are indistinguishable, but significant differences in performance set in once the level of zinc becomes limited (Genc et al., 2007). Similarly, the *brb* mutant is less able to take up cadmium, which leads to the conclusion that up to 45% of the uptake occurs through the RHs (Zheng et al., 2011).

Since RHs significantly increase root surface area, their role in water uptake is postulated by many authors; however, there is little experimental evidence to confirm this hypothesis. Segal et al. (2008) showed that only the tip domain of the RH is directly involved in water uptake in barley. The water potential of the soil lodged between adjacent RHs of cv Pallas has been shown to become equilibrated with that inside the root tissues within 1 min, after which time water uptake ceases (Segal et al., 2008). The same authors further showed that the efficiency of water uptake is 55% higher in wild-type roots than in those of the hairless mutant *brb*, thus reflecting the large root surface available for water uptake. On the other hand, no water-deficient features were observed for the root-hairless mutants *rhl1.a* to

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* Address correspondence to marek.marzec@us.edu.pl.

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rhl1.c when plants were grown under optimal conditions in a growth chamber (Chmielewska et al., 2014), giving the assumption that RHs are not essential for water uptake in well-watered soil. RH elongation is more pronounced in plants that are grown in dry soil than in those that do not experience any moisture stress. However, under combined drought and P-deficiency stresses, barley mutants impaired in RH growth showed a limitation in agronomy-important features, such as grain weight, number of grains, number of tillers, and aboveground biomass, in comparison with wild-type plants (Brown et al., 2012). It has been demonstrated that water availability may influence the length of the RHs. In the case of mutants with short RHs grown under drought conditions, the length of the RH was increased slightly, although it never reached the value observed in the wild type (Haling et al., 2014). The overall conclusion is that the increase of RH length may be an adaptive trait to drought stress, similar to nutrient deficiency.

In the dicotyledonous species soybean (*Glycine max*), proteomic analysis has identified that many transporters are involved in the uptake of water and mineral nutrients (Brechenmacher et al., 2012), and a similar scenario applies to Arabidopsis (*Arabidopsis thaliana*), where a root-hairless transgenic line, NR23, overexpressing the 23-residue N-terminal domain of the plasma membrane-associated Ca^{2+}-BINDING PROTEIN2 showed 47% reduction in water uptake under normal conditions and exhibited less drought tolerance in comparison with wild-type plants (Tanaka et al., 2014). The indications are that any enhancement in RH function may offer a potential route to improving the productivity and stress tolerance of crops that are grown in poor soils (Meister et al., 2014). Last, but not least, the root epidermis of grass species represents a useful model for cell differentiation (Marzec and Kurczynska, 2014).

"TO BE OR NOT TO BE, THAT IS THE QUESTION": ROOT EPIDERMIS PATTERNING

RH development is conventionally regarded as a three-phase process: the first stage sees the establishment of root epidermis patterning, which involves a population of trichoblasts and atrichoblasts; the second comprises the formation of the bulge on the root surface, presaging the formation of an RH tube; and finally, the RH tip begins to grow. The current understanding of root epidermis patterning in dicotyledonous plant species is largely based on the behavior of Arabidopsis, in which trichoblasts and atrichoblasts are arranged in rows. The key regulators of cell fate have been identified (Bruex et al., 2012; Grebe, 2012). There are three types of rhizodermis patterning in plants: (1) all epidermal cells may produce RHs; (2) trichoblasts and atrichoblasts are present in each rhizodermal cell file; and (3) cell files composed only of trichoblasts and atrichoblasts are present (Dolan, 1996). At least two modes of patterning have been documented in the grasses: in the first, every epidermal cell is capable of differentiating into an RH, while in the other, it is exclusively the shorter cells that are endowed with the dense cytoplasm that later emerge as trichoblasts (Clowes, 2000; Kim and Dolan, 2011). Marzec et al. (2014a) have proposed a more nuanced classification of cell epidermis patterning, which is based on observations of the final division of the epidermal cell. In this scheme, type I cells describe those in which the last division is symmetrical. Two type I subtypes can be distinguished: in subtype IA cells, trichoblasts can only be distinguished from atrichoblasts by the presence/absence of an RH tube, while subtype IB cells first divide symmetrically but then expand asymmetrically, with the larger daughter cells differentiating into trichoblasts and the smaller ones into atrichoblasts. In type II cells, the last division is asymmetrical: subtype IIA trichoblasts end up on the upper (shoot-ward) side, while subtype IIB trichoblasts finish on the lower (root-ward) side (Fig. 1).

The question of which genes are responsible for root epidermis patterning in grass species still remains open. Histological analyses of the root epidermis in barley, *Brachypodium distachyon*, and rice (*Oryza sativa*) have demonstrated some distinct species differences, thus indicating that the process is not rigidly conservative. In barley and rice, following the symmetric cell division, an as yet unidentified signal directs daughter cell differentiation (Kim and Dolan, 2011; Marzec et al., 2013), while in *B. distachyon*, certain extrinsic factors, which appear to be activated prior to the last cell division, exert control over daughter cell identity (Kim and Dolan, 2011). The implication is that any attempt to elucidate the mechanism based on the early stages of RH development should focus on the factors that are related to asymmetric cell division and expansion. A restriction in symplasmic communication is observed

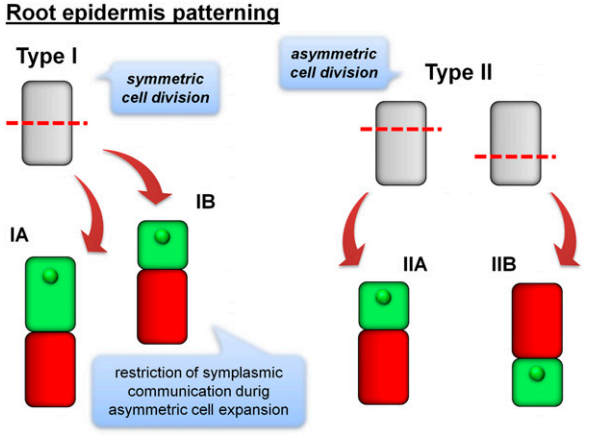

Figure 1. Two types of the establishment of root epidermis patterning in monocots. Type I is characterized by an initial symmetric division of a mother cell. In subtype IA, the two identical daughter cells do not show any differences, except for the presence or absence of an RH tube. In subtype IB, the two daughter cells develop into trichoblasts and atrichoblasts through asymmetric expansion, which correlates with a restriction of symplasmic communication between neighboring cells. In type II, the last division is asymmetric. A shorter cell may be located in the shoot-ward (subtype IIA) or root-ward (subtype IIB) position.

between neighboring cells during plant cell differentiation (Marzec and Kurczynska, 2014). In wild-type barley, symplasmic communication is limited to the root zone in which cells start to develop, whereas in the root-hairless mutant *rhl1.b*, all epidermal cells, even those in the mature zone of the root, remain interconnected; the absence of callose deposits in the plasmodesmata has been suggested as the basis for this difference (Marzec et al., 2014b). Apart from the allelic mutants that have been isolated in barley (Gahoonia et al., 2001; Chmielewska et al., 2014), hairless mutants have not as yet been obtained in the grasses. In rice and maize, some of the mutants that were classified previously as root hairless in fact develop very short RHs, while others exhibit bulges on the root that fail to develop into a recognizable RH (Hochholdinger et al., 2008; Yuo et al., 2009, 2011).

"RETURNING WERE AS TEDIOUS AS GO O'ER": RH INITIATION

Although root epidermis patterning differs between species, the staging of RH differentiation, beginning with bulge formation and ending with the elongation of the RHs, is universal (Fig. 2). The formation of the RH bulge has been well researched in Arabidopsis (Galway et al., 2011; Pei et al., 2012), but among the grasses, only a handful of mutants in which RH development has been arrested at this stage have been described. Even in these, the RH bulge is still present, as the mutant phenotype results from the inhibition of RH elongation (Szarejko et al., 2005). Only one mutant (*rth3* in maize) represents a genuine disturbance of bulge formation (Wen and Schnable, 1994). The target of this mutation encodes a protein that belongs to the grass clade of COBRA proteins (Hochholdinger et al., 2008). They harbor a glycosylphosphatidylinositol anchor that is attached to the C terminus and are thought to be involved in posttranslational modification and intracellular trafficking (Fujita and Kinoshita, 2012). The upregulation of *RTH3* in young primary roots and the localization of its transcription to the trichoblasts are consistent with the participation of these COBRA-like proteins in RH differentiation. Their mechanism of action remains unclear, since *RTH3* transcription can be detected almost throughout the maize plant, whereas at the phenotypic level, the mutant and the wild type only differ from one another with respect to RH development. The proposition is that the RTH3 protein contributes to cell wall synthesis and cell expansion and acts as a regulator of a small group of RH-specific genes (Hochholdinger et al., 2008).

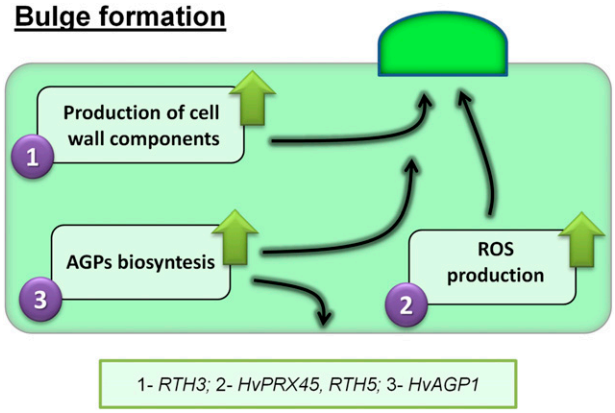

Figure 2. Mechanisms and genes that are involved in the formation of the RH bulge in monocots. This stage of development is related to three processes: (1) increased biosynthesis of cell wall components and their transport to the forming bulge; (2) higher activity of the enzymes that produce reactive oxygen species (ROS); and (3) production and subcellular transport of arabinogalactan proteins (AGPs).

Ten other genes that are potentially involved in RH development in barley were identified via a transcriptomic comparison between cv Karat and the *rhl1.a* mutant. Three of these encode peroxidases, two xyloglucan endotransglycosylases, one an arabinogalactan protein, one an extensin, one a Leu-rich repeat protein, one a phosphatidylinositol phosphatidylcholine transfer protein, and the last a RhoGTPase GDP dissociation inhibitor (Kwasniewski et al., 2010). The transcript abundance of all 10 genes is lower in the mutant than in the wild-type plant, while there is no transcriptional difference between the wild type and the *rhp1.b* mutant that produces an RH bulge. The products of all of these genes are related to either the cell wall or the plasma membrane, modifications to which may be key during bulge formation. The involvement of peroxidases in bulge formation could be confirmed by the detection of unusually high levels of ROS in the trichoblasts, especially in the cell wall of a bulge and at the tip of a growing RH (Kwasniewski et al., 2013a). Furthermore, roots harvested from seedlings that are grown in a medium containing an inhibitor of peroxidase develop a much lower density of RHs. Finally, in situ mRNA hybridization experiments have shown that the transcription of *HvPEROXIDASE45* is restricted to individual root epidermal cells (Kwasniewski et al., 2013a). In maize, the gene *RTH5* encodes a grass species-specific NADPH oxidase; its loss-of-function mutation resulted in a marked drop in both RH density and RH length (Nestler et al., 2014). The phenotype can be related to a reduced accumulation of ROS in the trichoblasts. Thus, it would appear that ROS, along with the enzymes that are required for their production, are necessary for RH differentiation to occur in the grasses, as is also the case in Arabidopsis (Huang et al., 2013a; Sundaravelpandian et al., 2013).

The possible involvement of AGPs in an early stage of RH development in barley, which is based on their nonhomogenous distribution in root epidermal cells at an early stage of specialization, was recently posited by Marzec et al. (2015). Treatment with a reagent that binds to all classes of AGP, and thereby strips the root surface of functional AGPs, has been shown to suppress RH expansion. The AGPs that are present in the extracellular matrix are believed to affect the organization of the

cortical microtubules that are involved in epidermal cell elongation (Nguema-Ona et al., 2007), whereas periplasmic AGPs may function as calcium capacitors (Lamport and Várnai, 2013). Considering the established role of AGPs in pollen tube elongation (Nguema-Ona et al., 2012), the proposition is that the AGPs are important for normal cell tip growth in barley and, hence, for all stages of RH development (Marzec et al., 2015).

"TIS WITHIN OURSELVES THAT WE ARE THUS OR THUS": ELONGATION OF THE RH TUBE

Studies of tip growth have featured strongly in analyses of RH development in the grasses (Fig. 3). Tip growth follows the elongation of the RHs or the pollen tubes (Rounds and Bezanilla, 2013). The establishment of a gradient in both ROS and Ca^{2+} is required to determine the direction of cell expansion, the reorganization of the cytoskeleton, and the vesicular transport of the cell wall components to the tip (Ketelaar, 2014; Zhou et al., 2014). Since rapid cell elongation requires a sufficient supply of cell wall building blocks, specifically cellulose, hemicellulose, and pectin, any distortion in their synthesis or transport will inevitably result in growth inhibition (Chebli et al., 2013). The earliest reported involvement of cellulose synthase in RH elongation in the grasses relates to the product of *OsCSLD1*, a rice gene that encodes a cellulose synthase-like D1 protein (Kim et al., 2007). This gene is expressed throughout the plant as well as in trichoblasts, atrichoblasts, and root cortex cells (Yuo et al., 2011). A *Dissociation element* (*Ds*) insertion mutant suffered a 65% to 75% reduction in RH length but no difference in RH density or distribution, which was taken to imply a role for OsCSLD1 in RH elongation. Confirmation was obtained from the behavior of an *OsCSLD1* overexpressor, which produced RHs that were double the length of those developed by wild-type plants (Kim et al., 2007). A second rice mutant, termed *rth2*, was shown to be a loss-of-function allele of *OsCSLD1* (Yuo et al., 2011). Unlike those of the *Ds* insertion mutant, *rth2* roots fail to develop bulges on the root surface. The gene copy in the former line is disrupted by the presence of a transposon close to the 3′ end of the first exon, which results in the production of a nonstandard transcript; in the latter, a premature stop codon is created close to the 5′ end of same exon. A similar situation occurs in Arabidopsis, where a mutated form of the ubiquitously expressed gene *CSLD3* severely inhibits RH elongation, although it does not compromise the formation of the bulge (Wang et al., 2001). Defects in *OsCLDS1* or *AtCSLD3* are generally complemented by various paralogs, although this is not the case for the development of RHs (Yuo et al., 2011). Moreover, a mutation in the *OsXXT1* gene, encoding xyloglucan 6-xylosytransferase, results in abnormal RH elongation. The product of *OsXXT1* is involved in establishing the cellulose-xyloglucan network, and a mutant phenotype indicates that this network is crucial in the cell wall formation during RH elongation (Wang et al., 2014).

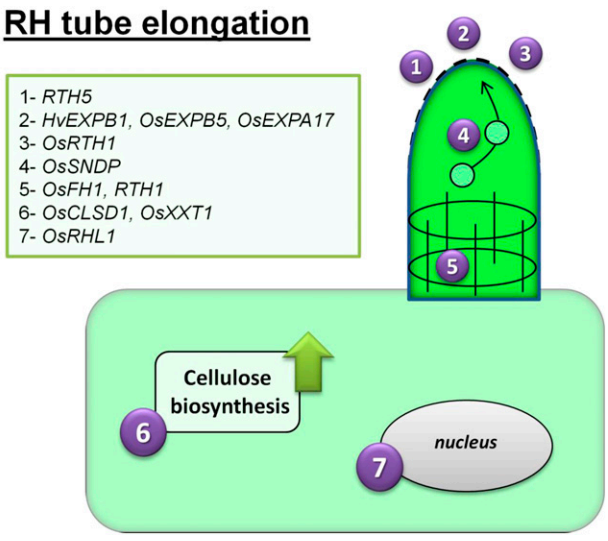

Figure 3. Scheme of RH tube elongation in monocots. The following seven processes are involved in tip growth: (1) loosening of the cell wall by expansins; (2) vesicle transport of cell wall components such as cellulose to the tube tip; (3) extracellular ATP hydrolysis by apyrases, which leads to their accumulation on the RH tip; (4) and (5) reorganization of the cytoskeleton is related to vesicle transport and tube elongation; (6) induction of cellulose synthesis, its transport to the growing RH tube, and formation of the cellulose-xyloglucan network; and (7) a transcription factor with a basic helix-loop-helix (bHLH) domain is active in the nucleus of the RH cell.

The cell wall components that are required for RH elongation are transported to the growing tip via vesicle transport and exocytosis to the extracellular matrix (Lombardo and Lamattina, 2012). To date, the only RH gene that is related to exocytosis that has been identified in the grasses is *RTH1*, a maize gene that encodes a homolog of the subunit of exocyst3 (SEC3). While a mutation in this gene induces a major shortening of RH length, it has no effect on pollen tube elongation, thus suggesting an RH-specific function for RTH1 (Wen et al., 2005). In Arabidopsis, a mutation in the homolog *EXO70A1* results in a negative pleiotropic effect on both RH length and plant organ size (Synek et al., 2006).

A second major group of proteins that have been determined to be related to tip growth are the expansins, which help to loosen the cell wall during tube elongation (Gu and Nielsen, 2013). Three expansins, which are encoded by *HvEXPB1* (Kwasniewski and Szarejko, 2006), *OsEXPB5*, and *OsEXPA17* (Won et al., 2010; ZhiMing et al., 2011), have been proven to be involved in RH initiation and elongation. Mutant analysis showed that the *HvEXPB1* transcript is present in the root but not in the shoot (Kwasniewski and Szarejko, 2006). The RH-specific cis-element features in the promoters of both *HvEXPB1* and *OsEXPB5*. Promoter experiments have demonstrated that both are active in an RH-specific manner when they are expressed in either Arabidopsis or rice and, more specifically, both at an early stage of bulge formation and during RH

elongation (Won et al., 2010). A rice line that produced RHs that were 70% shorter than those of the wild type has been shown to harbor a single nucleotide variant for *OsEXPA17*. This gene is specifically transcribed in the RH, and its promoter also includes an RH-specific cis-element (ZhiMing et al., 2011). The mutant phenotype proved to be partially reversible by constitutively expressing *OsEXPA30*, which is a gene encoding an RH-specific expansin. A recent report showed that the overexpression of *OsEXPA8* increases the length of rice RHs, primary roots, and the number of lateral roots that are formed (Ma et al., 2013).

In addition to the contribution of cellulose synthases and expansins to RH tip growth, some regulatory genes have also been identified. Among these is *OsRHL1*, a rice gene encoding a bHLH transcription factor, the overexpression of which increases RH length (Ding et al., 2009). Apart from producing foreshortened RHs, no other discernible phenotype could be associated with its loss of function. The gene is transcribed strictly in the trichoblasts, but surprisingly, its product localizes to the nucleus, thereby indicating a regulatory function. The participation of the bHLH transcription factors in RH elongation was also noted in both *Lotus japonicus* (*LjRHL1*) and Arabidopsis (*AtLRL1* to *AtLRL3*; Karas et al., 2009). Mutants for each of the three Arabidopsis genes are associated with an RH phenotype that is similar to that of *Osrhl1*, which is involved in the inhibition of RH development at the bulge stage. LjRHL1 deposition is restricted to the root epidermis nucleus, which has been taken to indicate a universal bHLH-mediated mechanism for the regulation of RH elongation (Karas et al., 2009).

Analysis of another rice mutant that develops greatly foreshortened RHs led to the identification of *OsRTH1* (encoding an apyrase), the product of which exerts a pleiotropic effect over plant stature, seminal root length, and the outgrowth of root bulges. The ATP content of the mutant's roots is double that present in the wild-type root (Yuo et al., 2009), a finding that may explain the disruption to RH tip growth, since some of the ATP that is hydrolyzed by apyrase activity is used for cell growth (Roux and Steinebrunner, 2007; Wu et al., 2007). In Arabidopsis, barrel medic (*Medicago truncatula*), and wheat, ATP was localized within the extracellular matrix of the tip of a growing tube (Kim et al., 2006). The proposition is that an unequal distribution over the RH surface of this ATP is essential for the establishment of the ROS and Ca^{2+} gradients that are required for RH elongation (Choi et al., 2014).

Phosphoinositide is a particularly important signaling molecule in the context of RH elongation. Disorders in its metabolism can lead to defective RH development, including either their foreshortening and/or branching (Heilmann, 2009; Yoo et al., 2012). Although the involvement of phosphoinositide in RH development has been well studied in Arabidopsis (Kusano et al., 2008; Stenzel et al., 2008), to date, only one gene, rice *Sec14-Nodulin Domain-Containing Protein1* (*OsSNDP1*), has been implicated in this process in the grasses. This gene encodes a phosphatidylinositol transfer protein (Huang et al., 2013b). As in the Arabidopsis *Sec fourteen homologs1* mutant (Vincent et al., 2005), the RHs that are formed by the *Ossndp1* mutant are shorter than those of the wild type and frequently form branches. This phenotype suggests a severe disorder with respect to polar growth and probably reflects cytoskeleton disorganization and/or a disturbed vesicle transport. Since the overexpression of *OsSNDP1* has no impact on RH length, the likelihood is that its product is not directly involved in RH elongation but, rather, in the determination of the direction of tube expansion (Huang et al., 2013b).

A final group of proteins that are implicated in RH development in the grasses is the formins. These proteins are involved in cell division and organ expansion as well as in tip growth (Yang et al., 2011; Wang et al., 2012). They are also important for actin polymerization and, thus, in the organization of the cytoskeleton (van Gisbergen and Bezanilla, 2013). An analysis of a short RH rice mutant revealed a point mutation in the gene rice *Formin Homology1* (*OsFH1*), which encodes a formin-like protein (Huang et al., 2013c). The expression of a mutant phenotype is environmentally dependent, however. In plants that are raised in a liquid medium, the RH of the mutant is somewhat shorter than that of the wild type, but when the seedlings are raised on a solidified medium, this difference disappears. Treatment with either auxin or ethylene, or the exposure of the plants to either P or iron starvation, has no effect on *Osfh1* RH length. It has been suggested that the roots of the mutants may be more sensitive to oxygen depletion than the wild-type roots, a condition that tends to prevail in liquid cultures (Huang et al., 2013c). Nevertheless, this is, to our knowledge, the first report of a mutation in a gene that is associated with formin affecting RH elongation; in doing so, it confirms the importance of cytoskeleton organization in this process.

WHAT DO WE KNOW?

A focus on root traits has been a feature of research that is associated with the breeding of wheat (Wasson et al., 2012), barley (Brown et al., 2013a; Haling et al., 2013), and maize (Bayuelo-Jiménez et al., 2011). Simultaneously, attempts have been made to uncover the molecular basis of RH development in the grasses at both the transcriptomic (Kwasniewski et al., 2010) and proteomic (Nestler et al., 2011; Janiak et al., 2012) levels. Mutants have provided an invaluable means for identifying the key genes that are involved in the various stages of RH formation as well as to validate conclusions that are based on large-scale analyses (Chmielewska et al., 2014). Meanwhile, histological analyses of cell modifications during RH development have led to a better understanding of the differentiation process (Kim and Dolan, 2011; Marzec et al., 2013, 2014b). The use of confocal laser scanning, transmission, and scanning electron microscopy has enabled a number of insights to be gained into how the RHs behave under controlled experimental conditions. A more novel development is

the deployment of synchrotron radiation x-ray tomographic microscopy to investigate RH function under natural conditions (Keyes et al., 2013). Gradually, a fuller picture of the RH differentiation process is being assembled (Table I).

WHAT WE STILL NEED TO KNOW

Although the last few years have seen significant progress being made in uncovering the mechanisms that are involved in RH differentiation in the grasses, major knowledge gaps still persist, especially as compared with the much fuller information that is available for Arabidopsis, in which 138 genes related to various stages of RH development have already been identified (www.iroothair.org; Kwasniewski et al., 2013b). Some commonalities have been established between monocotyledonous and dicotyledonous species, but major differences in the initial rhizodermis specialization step remain. While 21 genes that are involved in the production of trichoblasts and atrichoblasts have been defined in Arabidopsis, the number of grass species genes that are related to the early stage of rhizodermis differentiation is still zero. Additionally, there appear to be significant differences between the various grasses with respect to trichoblast/atrichoblast formation. At present, recognition of the molecular mechanisms that underlie rhizodermis patterning in grasses is the most pressing research priority. Achieving this should be possible through a combination of molecular, cytological, and histochemical studies that are targeted at RH mutants.

CONCLUSION

Continued progress in crop plant improvement implies the development of cultivars that are better adapted to drought stress and low soil fertility. Given that the roots represent the front line for both water and nutrient uptake, it is logical to focus resources on improving the root system (Lynch, 2011; White et al., 2013). While root traits are not easy to quantify, both the density and length of RHs are readily measurable and are likely to be highly correlated with a plant's ability to take up water and minerals from the soil (Brown et al., 2013a; George et al., 2014). Thus, a greater knowledge of the molecular basis of RH differentiation offers the potential to make significant advances that will be relevant to growing the crops of the future. At the same time, the importance of a plant's performance in a field situation should not be underestimated. Recent data indicate that, in some cases, the perceived genetic differences in RH length, which were concluded from laboratory-based experiments, can be modified when the plants are exposed to drought stress or nutrient deficiency (Haling et al., 2013, 2014). The take-home message is that the development of improved cultivars cannot be based on the performance of single mutants or on the modification of single genes or proteins, unless perhaps the

Table I. *Genes that are involved in RH development in monocots*

Gene	Encoded Protein	Protein Function	Species	Mutant	References
Stage of RH development: bulge formation					
RTH3	COBRA-like protein	Cell expansion, cell wall components, biosynthesis	Maize	+	Hochholdinger et al. (2008)
HvPRX45	Peroxidase	ROS production	Barley	−	Kwasniewski et al. (2013a)
Stage of RH development: tip growth					
RTH5	NAPDH oxidase	Establishment of a high level of ROS in RH tips	Maize	+	Nestler et al. (2014)
HvEXPB1	Expansin	Cell wall loosening	Barley	−	Kwasniewski and Szarejko (2006); Won et al. (2010)
OsEXPB5	Expansin	Cell wall loosening	Rice	−	Won et al. (2010)
OsEXPA17	Expansin	Cell wall loosening	Rice	+	ZhiMing et al. (2011)
OsRTH1	Apyrase	Hydrolyzation of extracellular ATP	Rice	+	Yuo et al. (2009)
OsSNDP	Sec14-nodulin domain-containing protein	Vesicle transport and organization of the cytoskeleton	Rice	+	Huang et al. (2013c)
RTH1	Exocyst subunit SEC3	Polar exocytosis	Maize	+	Wen et al. (2005)
OsFH1	Formin-like protein	Polymerization of the actin cytoskeleton	Rice	+	Huang et al. (2013b)
OsCLSD1	Cellulose synthase-like D1 protein	Synthesis of cellulose	Rice	+	Kim et al. (2007); Yuo et al. (2011)
OsXXT1	Xyloglucan 6-xylosytransferase	Formation of the cellulose-xyloglucan network	Rice	+	Wang et al. (2014)
OsRHL1	bHLH transcription factor	Function unknown	Rice	+	Ding et al. (2009)

gene/protein that is concerned plays a regulatory role over root epidermis differentiation.

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