The evolutionary context of root epidermis cell patterning in grasses (Poaceae)

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In the last century, the mechanism for establishing the root epidermal pattern in grasses was proposed as a differentiating trait that can be used in taxonomic studies and as a useful tool to indicate the relationships between genera. However, knowledge about root hair differentiation in monocots is still scarce. During the last few years, this process has been studied intensively, mainly based on genetics and histological studies. A histological analysis of the root epidermis pattern composed from root hairs (trichoblasts) and non-root hair cells (atrichoblasts), as well as observations of the mechanism of the establishment of this pattern allowed 2 different methods of epidermal cell specialization in monocots to be precisely described. Additionally, a recently published paper describing root hair development in barley shed new light on the evolutionary context of the mechanism of root epidermis cell specialization, which is discussed in the presented work.

In dicots, the root epidermis with the exception of the presence of root hairs, whereas in both the second and the third epidermal cell pattern, the cells that produce root hairs are shorter, have a denser cytoplasm and a reduced vacuolation in comparison to the non-root hair cells. These differences allow trichoblasts to be distinguished from atrichoblasts, even in the meristematic zone.

There are 2 main ways that unequal root epidermal cells originate—an asymmetric division of a mother cell or an asymmetric expansion of daughter cells after a symmetric division. Asymmetric divisions play a crucial role in plant cell differentiation and create cellular diversity—2 unequal daughter cells may start to realize different developing programs directly after the division. This happens because the daughter cells differ not only in size but also in their shape, localization, composition, and the number of organelles, the amount of cytoplasm and molecules that are localized in the cytoplasm, such as proteins or RNAs. These types of cell divisions lead to the creation of new tissues/organs and are required for many developmental processes, among them plant embryogenesis, stomata development, and root epidermis patterning. In the case of the root epidermis, after the asymmetric division, the shorter daughter cell will develop into a non-root hair cell, whereas the longer cell will develop into a root hair cell. The shorter cell may be localized closer to the root meristem (called a rootward cell) or closer to the shoot meristem (called a shootward cell).

However, it is also possible that the shootward-last division of a root epidermal cell is symmetric, but that the 2 identical daughter cells start to expand asymmetrically. After a period of asymmetric growth, the daughter cells differ in size, cytoplasm density, the level of vacuolation, and the amount of mitochondria. Only the shorter epidermal cells can produce root hairs, whereas the longer cells become atrichoblasts.

The vast majority of our knowledge about root hair development comes from studies on dicotyledonous species,
especially *Arabidopsis thaliana*, whereas information about monocots is scarce. During the last few years, new genes that are involved in the root hair development in *Oryza sativa*\(^{16,17}\) and *Zea mays*\(^{18}\) have been identified and detailed histological studies on the root epidermis of *Brachypodium distachyon*, *rice*,\(^{10}\) and *Hordeum vulgare*\(^{11,19}\) have been performed. Moreover, some comparative analyses of the root epidermal pattern in grasses (Poaceae) were performed in the last century and the application of this kind of analysis for taxonomic studies was proposed.\(^{20-22}\)

The first report, which was published in 1939, described 2 types of the shootward-last divisions in Poaceae—type A, when the last division produces unequal daughter cells and type B, when daughter cells after the last division are identical.\(^{20}\)

Type A was observed in *Phleum pratense* L. and *Poa trivialis* L. (currently classified in the Pooideae subfamily);\(^ {23}\) type B when the daughter cells were indistinguishable in size and content was described in *Sporobolus cryptandrus* (Torr.) Gray (currently classified in the Chloridoideae subfamily) (Fig. 2, Table 1).\(^ {23}\)

The root epidermal cells of *Chloris gayana* Knuth. (currently classified in the Chloridoideae subfamily)\(^ {23}\) was described as an intermediate type between types A and B (Table 1) because the last division appeared to be slightly unequal.\(^ {20}\) In subsequent reports, 6 species (*Phalaris arundinacea* L., *Poa pratensis* L, *Phleum pretense* L., *Agrostis alba* L., *Agrostis tenuis* Sibth., and *Beckmannia syzigachne* Steud.) from the Pooideae subfamily (according to the current classification)\(^ {23}\) were classified as type A,\(^ {21,24}\) whereas representatives of the current Panicoideae subfamily (*Panicum capillare* L., *Digitaria sanguinalis* L., *Miscanthus sinensis*, *Andropogon scoparius* Michx.) showed the B type of cell epidermis divisions (Fig. 2; Table 1).\(^ {21}\)

According to the taxonomy of the previous century, any species representing type A of the root epidermis was classified into the Festucoideae subfamily and species expressing type B were classified into the Panicoideae subfamily, thus the type A was renamed as a “festucoid type” and type B as a “panicoid type.”\(^ {21}\)

All data that was presented indicate that the development of root hairs in Poaceae was considered to be an important taxonomic feature that correlated with other taxonomic characteristics, such as the number and size of chromosomes, embryo structure or leaf anatomy.\(^ {20,21,24}\) During the next large-scale analysis of monocot species in which 82 species were studied, one of the main features of root morphology was the presence of alternating long and short cells, but the study did not investigate the shootward-last

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**Figure 1.** Root epidermis patterning in plants. (A) Type I in which any root epidermal cell may produce a root hair, (B) Type II in which only the shorter cells produce root hairs, (C) Type III with a striped pattern in which files of root hairs and non-root hair cells are present. (D–E) Two possibilities for the presence of the alternative pattern of shorter and longer cells—(D) the asymmetric shootward-last division or (E) the asymmetric expansion of identical daughter cells after the symmetrical division.

**Figure 2.** Phylogeny of Poaceae from the Catalogue of New World Grasses. Blue indicates subfamilies in which symmetric shootward-last divisions is present, red indicates asymmetric shootward-last divisions, subfamilies that were not investigated are marked with white, the number of analyzed species can be find in brackets.
Table 1. Species of the Poaceae family with a known root epidermis pattern

| Subfamily          | Species                  | Type         | Description                                                                 | Refs. |
|--------------------|--------------------------|--------------|-----------------------------------------------------------------------------|-------|
| Bambusoideae       | Pleioblastus chino Max   | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
| Chloridoideae      | Sporobolus cryptandrus   | Type B       | Symmetric last division, some epidermal cells produce root hairs            | 20    |
|                    | Chloris gayana Kth.      | Type A/B     | Slightly asymmetric division, mainly shorter cells produce root hair         | 20    |
| Ehrhartoideae      | Oryza sativa L.          | Symmetric    | Symmetric shootward-last cell division, however after root hair initiation atrichoblasts elongate more than trichoblasts | 10    |
|                    | Agrostis alba L.         | Type A/festucoid | Asymmetric last division, only shorter cells produce root hairs              | 21    |
|                    | A tenus Sath.            | Type A/festucoid | Asymmetric last division, only shorter cells produce root hairs              | 21    |
|                    | Anisantha sterilis L.     | Vd           | Asymmetric shootward-last division, only shorter rootward cells produce root hairs | 25    |
|                    | Avena sativa L.          | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
|                    | Beckmannia crysophene Steud. | Type A/festucoid | Asymmetric last division, only shorter cells produce root hairs              | 21    |
|                    | Brachypodium sylviacum L. | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
| Pooidae            | B distachyon L.          | Asymmetric   | Asymmetric shootward-last division, only shorter cells produce root hairs   | 10    |
|                    | Elytrigia repens L.      | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
|                    | Festuca rubra L.         | Vd           | Asymmetric shootward-last division, only shorter rootward cells produce root hairs | 25    |
|                    | Holcus lanatus L.        | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
|                    | Hordeum vulgare L.       | Symmetric division | Symmetrical shootward-last division, asymmetrical expansion, only shorter, rootward cells produce root hairs | 15    |
|                    | Phalaris arundinacea L.  | Type A/Vd    | Asymmetric last division, only shorter rootward cells produce root hairs     | 21, 24, 25 |
|                    | Phleum pretense L.       | Type A/festucoid/Vd | Asymmetric last division, only shorter rootward cells produce root hairs     | 20, 21, 25 |
|                    | Poa trivialis L.         | Type A       | Asymmetric last division, only shorter cells produce root hairs              | 20    |
|                    | P pratensis L.           | Type A/festucoid | Asymmetric last division, only shorter cells produce root hairs              | 21    |
|                    | P annua L.               | Vd           | Asymmetric shootward-last division, only shorter rootward cells produce root hairs | 25    |
|                    | Secale cereal L.         | Vd           | Asymmetric shootward-last division, only shorter rootward cells produce root hairs | 25    |
|                    | S cereal x Triticum monococcum | N  | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
|                    | S cereal x T a estivum    | Vd           | Asymmetric shootward-last division, only shorter rootward cells produce root hairs | 25    |
|                    | T monococcum L.          | Vd           | Asymmetric shootward-last division, only shorter rootward cells produce root hairs | 25    |
|                    | T aestivum L.            | Vd           | Asymmetric shootward-last division, only shorter rootward cells produce root hairs | 25    |
| Panicoidae         | Panicum capillare L.     | Type B/panicoid | Symmetric last division, some epidermal cells produce root hairs            | 21    |
|                    | Digitaria sanguinalis L. | Type B/panicoid | Symmetric last division, some epidermal cells produce root hairs            | 21    |
|                    | Miscanthus sinensis A.   | Type B/panicoid | Symmetric last division, some epidermal cells produce root hairs            | 21    |
|                    | M sinensis L.            | Vd           | Asymmetric shootward-last division, only shorter rootward cells produce root hairs | 25    |
|                    | Andropogon scoparius Michx. | Type B/panicoid | Symmetric last division, some epidermal cells produce root hairs            | 21    |
|                    | Panicum miliaceum L.     | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
|                    | P capriere L.            | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
|                    | 2za mays L.              | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |
|                    | Saccharum officinarum L. | N            | No evident trichoblasts, symmetric shootward-last division, any cell can produce root hairs | 25    |

A description is given in the text; Vp—trichoblasts in vertical patterns and derived from the proximal sister cell; Vd—trichoblasts in vertical patterns and derived from the distal sister cell; N—no evident trichoblasts.

Another large-scale analysis, this one focused on the root meristem, was published in 2000. Authors described root hair development in some of plants as “intermediate” when classification as a festucoid or panicoid type was impossible. This report indicated the importance of root hair development as a useful feature in taxonomic studies; the authors identified species that should be removed from the described tribes and these recommendations were in accordance with the currently accepted phylogenetic classification.23

We now know that the occurrence of asymmetric cells may be caused by asymmetric divisions or asymmetric expansions of daughter cells after a symmetric division. Authors described root hair development in some of plants as “intermediate” when classification as a festucoid or panicoid type was impossible. This report indicated the importance of root hair development as a useful feature in taxonomic studies; the authors identified species that should be removed from the described tribes and these recommendations were in accordance with the currently accepted phylogenetic classification.23
correspond to the festucoid type and the third type (N), which has no evident trichoblasts, corresponds to the panicoid type. In this report, 21 species from the Poaceae family (mainly from the Pooideae subfamily) were analyzed. However, all of the studies were performed using simple histological stainings, which may generate errors, especially in the interpretation of symmetric and asymmetric divisions. For example, Clowes described *B. sylvaticum* and *H. vulgare* as type N species with no evident trichoblasts present, whereas studies conducted later, using confocal and electron microscopy, clearly showed that the trichoblasts and atrichoblasts could be distinguished in both species.

In the work of Kim and Dolan, techniques including, e.g., confocal laser scanning microscopy and cryo-scanning electron microscopy were used for the root analysis of 2 species—rice (from the Ehrhartoideae subfamily) and *B. distachyon* (from the Pooideae family). An asymmetrical shootward-last division was observed in rice and after root hair initiation, trichoblasts elongated more slowly than non-root hair cells, whereas in *B. distachyon* shorter cells localized in rootward position after the asymmetric division and started to develop root hairs. Based on their own results and literature data, the authors proposed the hypothesis that the asymmetric division evolved in the Pooideae or in an ancestor of Pooideae because only in this subfamily is the asymmetric division present. However, our work on root epidermis differentiation in barley (Pooideae subfamily) clearly indicated that the shootward-last division of root epidermal cells is symmetric and after this division the daughter cells immediately start to elongate asymmetrically. Only the shorter cells produce root hairs in the differentiation zone and more than 70% of the shorter epidermal cells are alternately arranged into trichoblasts and atrichoblasts. A pattern in which 2 or 3 shorter cells were adjacent to each other was also observed. The differences between trichoblasts and atrichoblasts were visible immediately after cell divisions, before root hair initiation. The shorter cells contained a denser cytoplasm, a greater number of mitochondria and only this type of cells produced root hair tubes. Differentiation of both cell types is correlated with the restriction of symplasmic communication between neighboring cells.

The presented results and literature data indicate that both types of root epidermis differentiation, which result from symmetric and asymmetric divisions, are present among representatives of the Chloridoideae, Pooideae, and Panicoideae subfamilies (Fig. 2; Table 1). However, as was mentioned above, some of the studies that have been performed recently using advanced cell biology techniques, such as confocal laser scanning microscopy or transmission/scanning electron microscopy have shown that several species have been described erroneously. This is why we postulate the necessity of a more sophisticated analysis of other species and re-examination of plants investigated with the simple microscopy techniques in the future. At present, we suggest using a modified nomenclature for a detail description of root epidermis differentiation (Fig. 3). Type I, when a symmetric shootward-last division is present, corresponding to type B of Sinnott and Bloch, the panicoid type of Reeder and von Maltzahn, and the N type of Clowes, which should be divided into 2 subtypes—IA and IB. In the IA subtype, both daughter cells do not show any morphological differences except for the presence or absence of root hair tubes and any epidermal cell may develop a root hair. The second subtype, IB, should group species in which a symmetric shootward-last division is present, but the differences between daughter cells become visible during differentiation and only shorter cells can produce root hairs, as has been described for rice and barley. Type II, which was originally described by the presence of shorter and longer cells, should be defined as a type in which the asymmetric shootward-last division occurs and should also be divided into 2 subtypes. In subtype IIA, corresponding to the Vp type of Clowes, a shootward daughter cell produces root hairs after the asymmetric division and IIB, in which only a rootward daughter cell differentiates into a trichoblast (corresponding to the Vd type of Clowes). Type III, in which files of trichoblasts and atrichoblasts are present, should remain unchanged (Fig. 3). The proposed classification allows the development of the root epidermis to be described based on the crucial mechanism in this process—the shootward-last division—and subsequently the processes that occur in daughter cells after this division. Now, we know that both asymmetric division and asymmetric cell expansion have been observed in the Chloridoideae, Pooideae, and Panicoideae subfamilies, which indicates that the mechanism of trichoblast/atrichoblast differentiation is not evolutionally conserved across the subfamilies of the Poaceae (Fig. 2; Table 1). However more studies on a larger scale and a re-examination of the literature data that was obtained using simple microscopic techniques are required to describe the evolution of root epidermis patterning in the Poaceae family.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
This research was supported by grants of the Polish National Science Centre: 2011/01/M/NZ2/02979 and 2013/08/T/NZ3/00811.
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