

Evaluation and Licensing Opportunities

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Patent Literature

International Patent Publication No. WO2008/142364
 Applications pending in US, EU, CN, IN, BR, CA and AU.

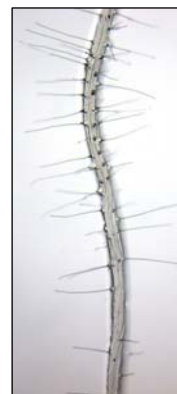
Root Hair Genes

RHD6 and RSL genes conserved in all land plants

Potential use for improved root structure giving enhanced water and nutrient uptake properties, better crop performance and yield

Professor Liam Dolan's group at the John Innes Centre have cloned and characterised basic helix-loop-helix transcription factors that control the development of root hairs in species as diverse as *Arabidopsis* and the bryophyte, *Physcomitrella patens*. The findings, first reported in *SCIENCE* (Menand et al, 2007) have significant implications for the understanding of land plant evolution as well as for crop improvement.

Root hairs are highly polarised cells that increase the surface area of the plant's root system, playing important roles in plant anchorage, water use and nutrient acquisition. *rhd6* mutants of *Arabidopsis* are "root hair deficient". The John Innes group cloned this gene and a family of related "RHD6-like" RSL genes and characterised their expression and effects in complementation studies. The AtRHD6 protein accumulates in hair-forming cells of the root epidermis causing root hairs to form, and acts on downstream of factors determining epidermal cell pattern formation, hence providing **a route to stimulate root hair formation directly**. Other RSL genes have also been characterised and are now known to play a central role in root hair development.



WT RHD6 gene transformed into mutant



rhd6 mutant

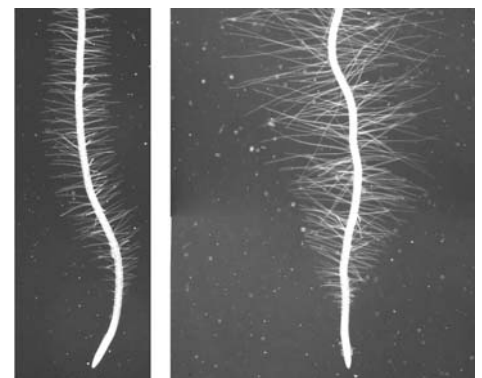


Wild type moss (L) and mutant lacking functional RHD6 homologue (R)

Liam Dolan's team have also studied *RSL* genes in other species, including the moss *Physcomitrella patens*, and by transformation into *Arabidopsis* showed that the moss *RSL* gene, PpRSL1 could substitute for the loss of *Arabidopsis* RHD6 function – suggesting that this technology will be **widely applicable across plant species**. As well as *Arabidopsis* and mosses, *RSL* genes have been identified in many species including: **rice, maize, wheat, barley, lotus, alfalfa**, and others.

In a *Nature Genetics* paper published February 2010, Prof. Dolan's group describe how:

- **RSL4 is the key regulator in the process of root hair growth**
- RSL4 is sufficient to promote growth of root hairs
- Constitutive expression of RSL4 results in **very long** root hairs
- When phosphate stress is sensed by the plant, RSL4 is induced
- As well as low phosphate, other factors that normally increase root hair growth, such as ethylene and auxin, all act via increased RSL4 transcript and protein levels



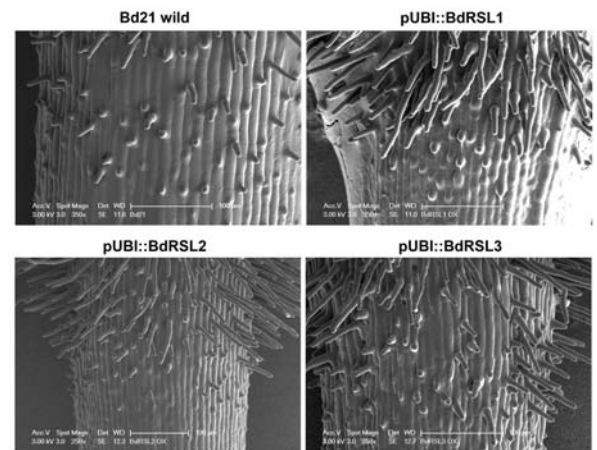
1 mm

In addition RSL genes have now been expressed in transgenic **brachypodium, rice and wheat**. In rice and brachypodium, endogenous orthologues of RSL4 were overexpressed using ubiquitin promoter. In both species this resulted in the formation of approx **3x longer root hairs**, and in brachypodium, also **more root hairs** are produced. In wheat, the Arabidopsis RSL4 was overexpressed and this results in longer and more root hairs. Reduction of RSL expression either by T-DNA mutation (brachypodium) or by RNAi downregulation (rice) results in only very short root hairs being formed. This is consistent with the understanding from Arabidopsis and moss, that RSL4 is the key regulator of root hair growth in land plants.



Low magnification images showing the increased root hair length in plants overexpressing Brachypodium genes in Brachypodium

Scanning electron micrographs of wild type roots (Bd21 wild) and three overexpressing events, showing that compared to wild type, the transgenic plants (1) develop more hairs develop and (2) have a greater rate of hair elongation.



Transformation of brassica is in progress.

The importance of root hairs in determining the performance of crop plants is as significant as it was for the first plants to colonise the land millions of years ago. The RHD6/RSL technology affords a direct route to developing crop plants with improved root properties either by **genetic transformation** or by, for example, **TILLING strategies** to create/select enhanced genetic variation for root-hair traits. Manipulation and improvement of root-hair formation may result in:

- better **uptake of nutrients** – in particular phosphate and iron, whose absorption is primarily via direct contact with root hairs
- better uptake/use of available soil moisture – and hence better **stress tolerance and yield**
- better physical anchorage of plants, better seedling establishment

There is also potential to use the technology to enhance production of valuable compounds in **root-hair culture systems**:

- natural phytochemicals - are known to be secreted by root hairs
- valuable heterologous compounds - in the context of “molecular pharming” applications.

The technology is assigned to and the subject of patent applications filed by PBL.

Patent applications are pending in USA, Europe, China, India, Brazil, Canada and Australia.

References:

Menand, Yi, Jouannic, Hoffman, Ryan, Linstead, Schaefer and Dolan (2007). An Ancient Mechanism Controls the Development of Cells with a Rooting Function in Land Plants. *SCIENCE*, 316 (5830): 1477-1480.

Keke Yi, Benoît Menand, Elizabeth Bell and Liam Dolan (2010). A basic helix-loop-helix transcription factor controls cell growth and size in root hairs. *NATURE GENETICS*, 42: 264-267.