Developmental Genetics of Root Hair Formation in *Arabidopsis thaliana*

Purpose: Examine the role that genes and the environment play in root hair patterning.

Background: Root hairs are small unicellular hair-like outgrowths that form from the surface of specific epidermal cells in the *Arabidopsis* root. The production of root hairs increases the surface area of the root available for nutrient and water acquisition. Their formation is under both genetic and environmental control. Today we will examine in optimal and sub-optimal nutrient conditions the expression of *GLABRA2 (GL2*), a marker of the non-hair cell fate, in three different mutants that affect the number and spatial patterning of root hairs.

Methodology: 1) Examine *GL2* expression in wildtype, *caprice (cpc)*,*werewolf* (*wer*) and *scrambled* (*scm*) mutant backgrounds.
2) Examine the effects of low phosphorus on root hair patterning in these lines.

Techniques Required/Learned: Media preparation, Use of a Laminar flow hood for aseptic plant tissue culture, Basic microscopy.

You will need:

- Microfuge tubes
- 3-5 day old seedlings grown on sterile MS/sucrose agar plates
  - WT GL2::GUS
  - *wer* GL2::GUS
  - *cpc* GL2::GUS
- GUS reagent (see below)
- Pipetman or transfer pipet
- Microfuge rotisserie (optional)
- Dissecting Scope
- Microscope slide
- Forceps
- Gloves
- 37˚ incubator (optional)

- Make the following GUS staining solution. *Larger volumes of GUS staining solution can be made, aliquoted and stored -20 C prior to experiment*:

100 ul	1M NaPO4 pH 6.8
20 ul	0.5 M EDTA pH 8.0
100 ul	5 mM potassium ferricyanide
100 ul	5 mM potassium ferrocyanide
5 ul	20% triton X-100
20 ul	20 mM X-Gluc RPI (www.rpicorp.com), catalog # B72200).
655 ul	distilled water
1ml total	

- Place seedlings in 1.65ml microfuge tubes and pipette enough GUS staining solution so seedlings are completely immersed. *The vessel size used for GUS staining can vary*

depending on the tissue size. Microtiter dishes can be used if assaying large numbers of samples.

- Incubate tissue in GUS staining solution for 37 C for 30 minutes to overnight depending on level of GUS activity in sample. For the GL2::GUS containing plants used in this experiment, staining should be present within 60 to 120 minutes. *Gentle agitation on a rotisserie can be used, or alternatively periodically mixing by hand should suffice.*
- Using forceps, place stained seedling on a microscope slide and observe staining pattern and root hair pattern using a dissecting scope.

Notes

- The GUS reporter gene is the bacterial ß-glucuronidase enzyme. The GUS enzyme catalyzes a reaction that uses the substrate X-gluc (5-bromo-4-chloro-3-indolyl glucuronide). The GUS reaction produces a colorless compound that upon oxidation forms a blue precipitate. Because the colorless compound diffuses, accurate staining requires rapid oxidation. K ferri/ferrocyanides promote oxidation.
- For viewing GUS expression in green tissue, removing the green color is important for analysis, especially for genes with low expression levels. To remove chlorophyll after GUS staining, multiple 30 minute ethanol (70%) washes can be performed until green pigment has been removed. Overnight incubation in 70% ethanol can also be carried out. Tissues can be stored in 100% EtOH for extended periods of time.
- For older tissue, vacuum infiltration may be required to get GUS reagent into all tissues.
- Stock storage: K ferri/ferro solutions should be stored in the dark at 4 C. X-gluc stored in the dark at –20 C.
- X-gluc should be made as a stock as needed in dimethylformamide.

Useful References:

1. Bernhardt, C., et al., *The bHLH genes GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) specify epidermal cell fate in the Arabidopsis root.* Development, 2003. **130**(26): p. 6431-9.
2. Bernhardt, C., et al., *The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the Arabidopsis root epidermis.* Development, 2005. **132**(2): p. 291-8.
3. Dinneny, J.R., et al., *Cell identity mediates the response of Arabidopsis roots to abiotic stress.* Science, 2008. **320**(5878): p. 942-5.
4. Guimil, S. and C. Dunand, *Patterning of Arabidopsis epidermal cells: epigenetic factors regulate the complex epidermal cell fate pathway.* Trends Plant Sci, 2006. **11**(12): p. 601-9.
5. **Guimil, S. and C. Dunand, *Cell growth and differentiation in Arabidopsis epidermal cells.* J Exp Bot, 2007. 58(14): p. 3829-40.**
6. Kirik, V., et al., *Functional diversification of MYB23 and GL1 genes in trichome morphogenesis and initiation.* Development, 2005. **132**(7): p. 1477-85.
7. Kirik, V., et al., *The ENHANCER OF TRY AND CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis.* Dev Biol, 2004. **268**(2): p. 506-13.
8. Kirik, V., et al., *ENHANCER of TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichome development of Arabidopsis.* Plant Mol Biol, 2004. **55**(3): p. 389-98.
9. Kwak, S.H. and J. Schiefelbein, *The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis.* Dev Biol, 2007. **302**(1): p. 118-31.
10. Kwak, S.H., R. Shen, and J. Schiefelbein, *Positional signaling mediated by a receptor-like kinase in Arabidopsis.* Science, 2005. **307**(5712): p. 1111-3.
11. Larkin, J.C., M.L. Brown, and J. Schiefelbein, *How do cells know what they want to be when they grow up? Lessons from epidermal patterning in Arabidopsis.* Annu Rev Plant Biol, 2003. **54**: p. 403-30.
12. Lee, M.M. and J. Schiefelbein, *Cell pattern in the Arabidopsis root epidermis determined by lateral inhibition with feedback.* Plant Cell, 2002. **14**(3): p. 611-8.
13. Marks, M.D. and J.J. Esch, *Initiating inhibition. Control of epidermal cell patterning in plants.* EMBO Rep, 2003. **4**(1): p. 24-5.
14. Ryu, K.H., et al., *The WEREWOLF MYB protein directly regulates CAPRICE transcription during cell fate specification in the Arabidopsis root epidermis.* Development, 2005. **132**(21): p. 4765-75.
15. Schiefelbein, J., *Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot.* Curr Opin Plant Biol, 2003. **6**(1): p. 74-8.
16. Simon, M., et al., *Distinct and overlapping roles of single-repeat MYB genes in root epidermal patterning.* Dev Biol, 2007. **311**(2): p. 566-78.
17. Wang, S., et al., *TRICHOMELESS1 regulates trichome patterning by suppressing GLABRA1 in Arabidopsis.* Development, 2007. **134**(21): p. 3873-82.

18. **Claire Grierson and John Schiefelbein (April 4 2002) Root Hairs. In CR Somerville, EM Meyerowitz, eds, The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD, doi: 10.1199/tab.0009, www.aspb.org/publications/arabidopsis/**

Seed Sterilization and Plating for root hair analysis

You will need:

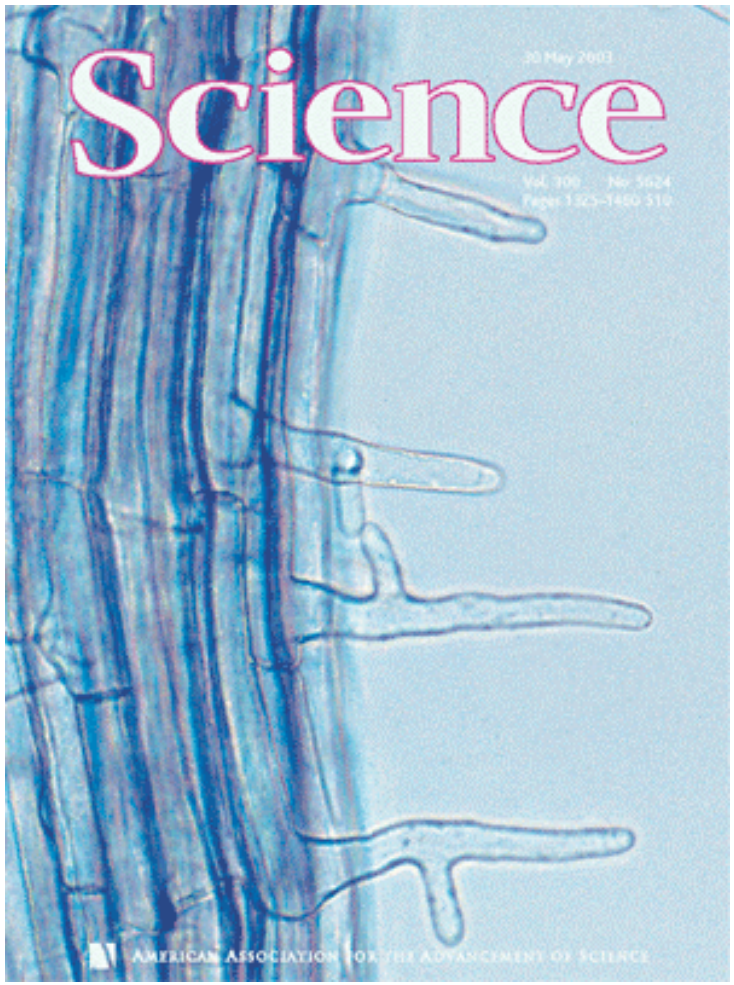
- Microfuge tubes
- Seed sterilization solution
- 70% Bleach (Standard bleach is 5.4% sodium hypochlorite. Check the ingredients in "germicidal" bleaches. These sometimes have other ingredients)
- 30% Distilled water
- 1 µl/ml 20% Triton X-100
- Seeds
- Sterile water
- Pipet
- MS media plates (or desired media) (pH 5.7 using KOH)
    - MS salts (Caisson MSP001*) 4.7 g/L
    - 1.0% granulated agar 10g/L
    - 0.05% MES Buffer 0.5g/L
    - 1% sucrose 10g/L

    Autoclave to sterilize and pour into petri dishes

- Place ~0.1 g of seeds into labeled microfuge tube.
- Add 500 uls of sterilization solution.
- Mix seeds by inverting tubes.
- Periodically vortex tubes over a 5.0 minute period – avoid over exposing seeds to sterilization solution.
- In a sterile hood, remove sterilization solution with pipet leaving the seeds in the tube. As an alternative to the use of a sterile hood for sterilization, this may be done in front of a lit Bunsen burner. The Bunsen burner will create an updraft that helps prevent airflow into the tube.
- Wash seeds at least 3x with 500 µl sterile water vortexing in between each rinse. A quick spin in a picofuge or microcentrifuge can help to get the seeds to settle between rinses.
- Add sterile water to the seeds and store seeds at 4°C for 1-2 days before plating.
- After at cold treatment, carefully pipette seeds in a row onto MS media.
- Leave plates open in sterile hood allowing excess water to evaporate.
- Place the plates under growth lights at 20-25°C. Incubate plates on side so roots grow on the surface of the media (seeds will stick to surface). Roots should appear in 1-2 days, and can be assayed in 3-5 days.

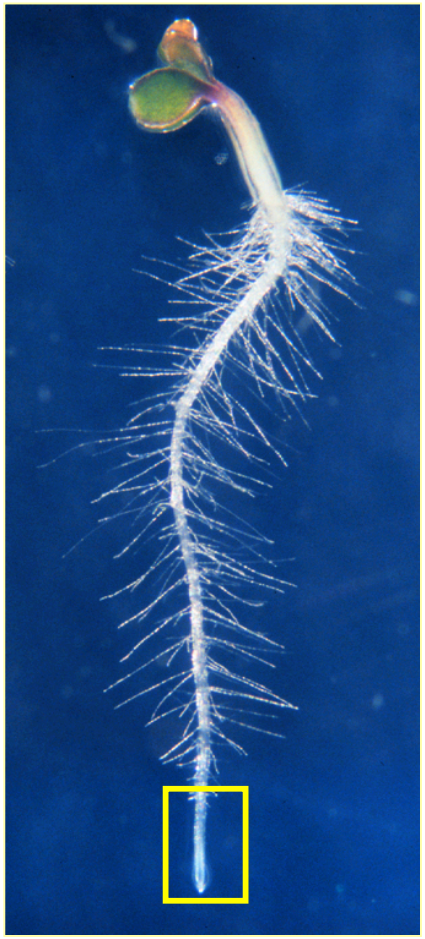
* Caisson is less expensive than Sigma for plant media reagents and in my experience their media are more reliable. Caisson also offers premixed MS media that lack specific nutrients like nitrogen or phosphorus. If you choose to use these as part of your lab course, make sure that the agar preparations that you use don't have micronutrients present.

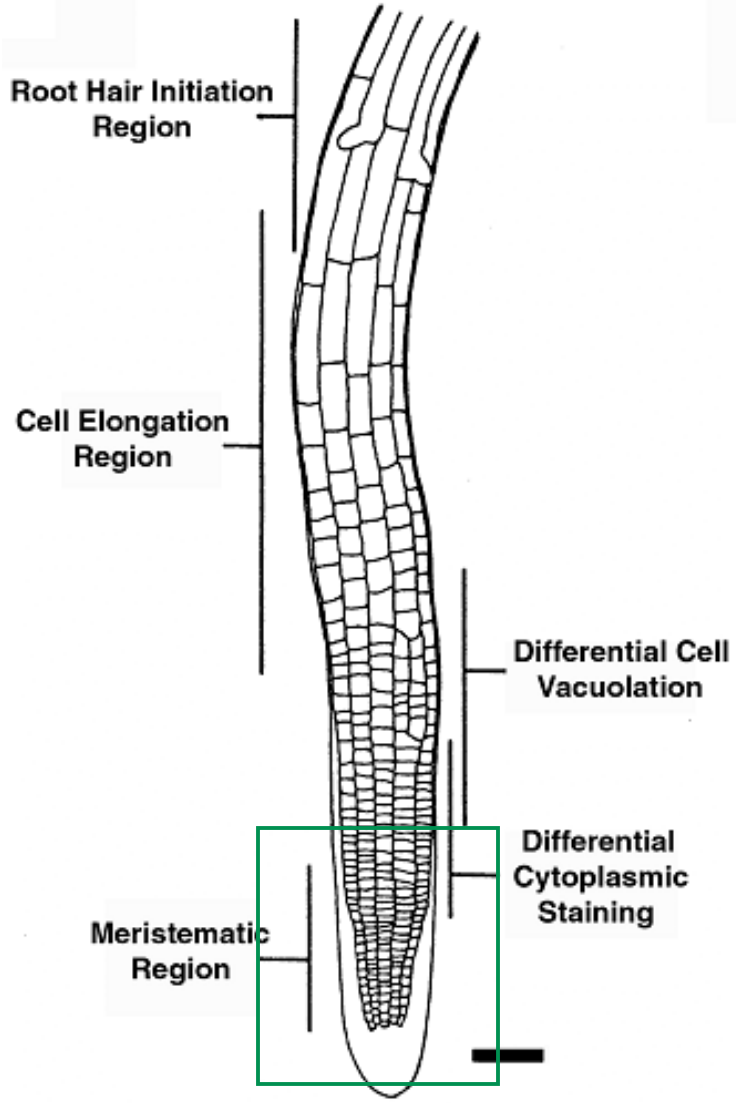
Developmental Genetics of *Arabidopsis* Root Hair Formation

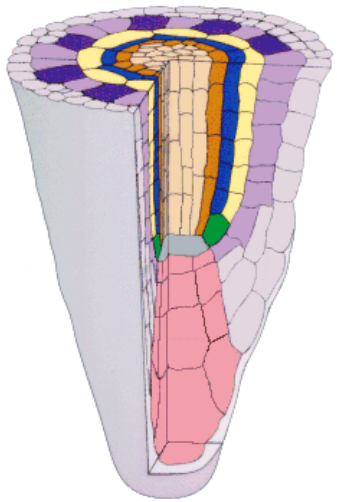


Modulation of Phospholipid Signaling by GLABRA2 in Root-Hair Pattern Formation. Yohei Ohashi, *et al.* Science 30 May 2003: Vol. 300. no. 5624, pp. 1427 - 1430

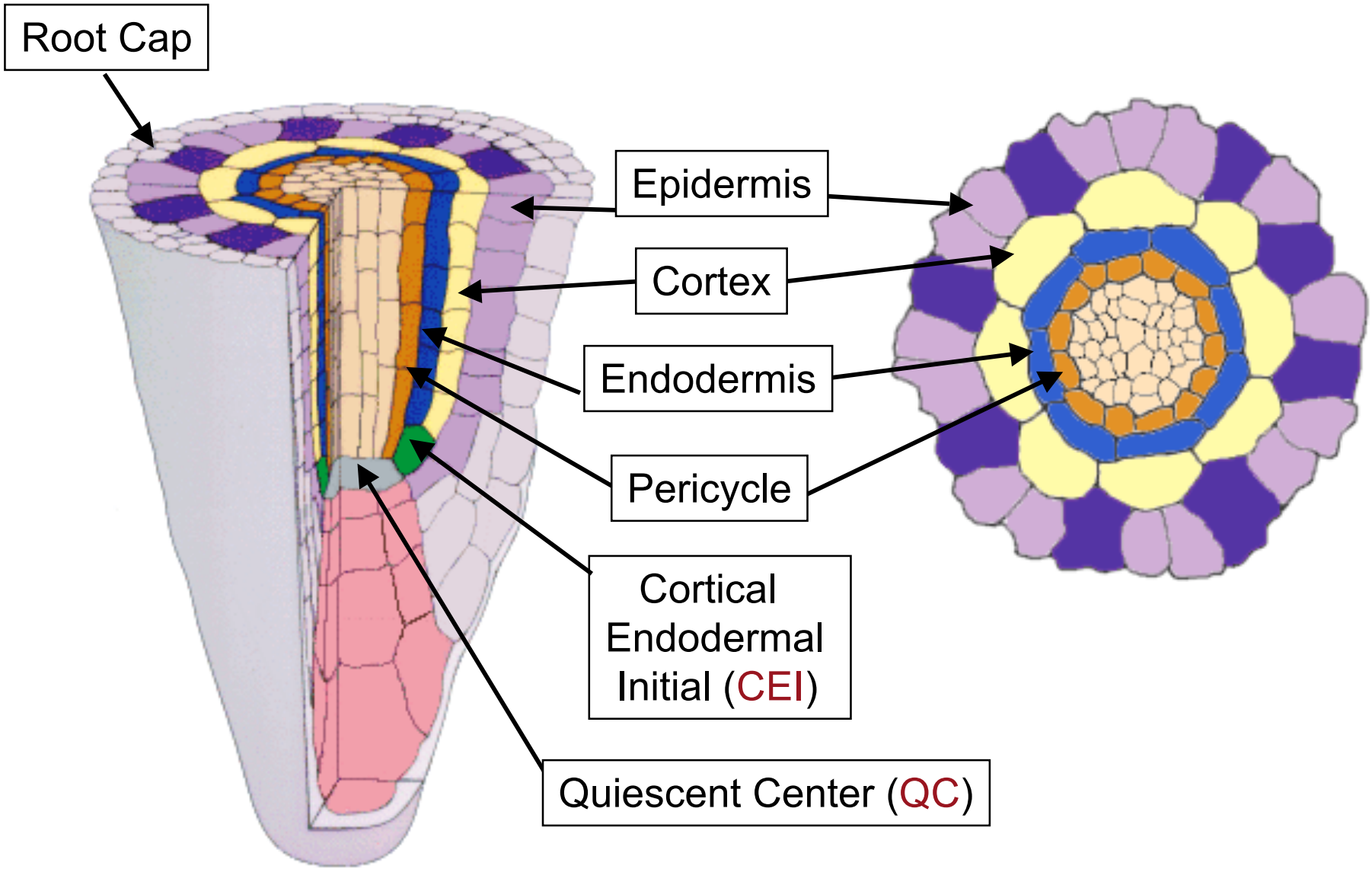
The *Arabidopsis* root



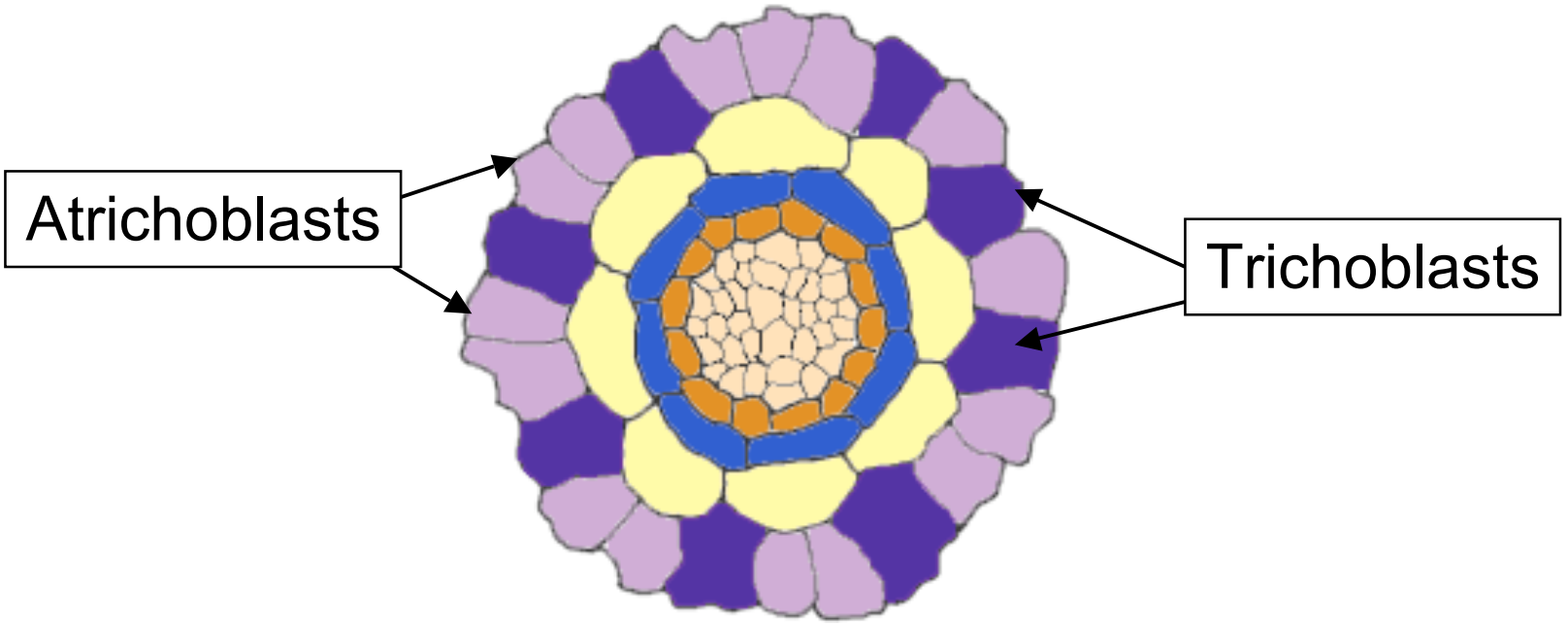




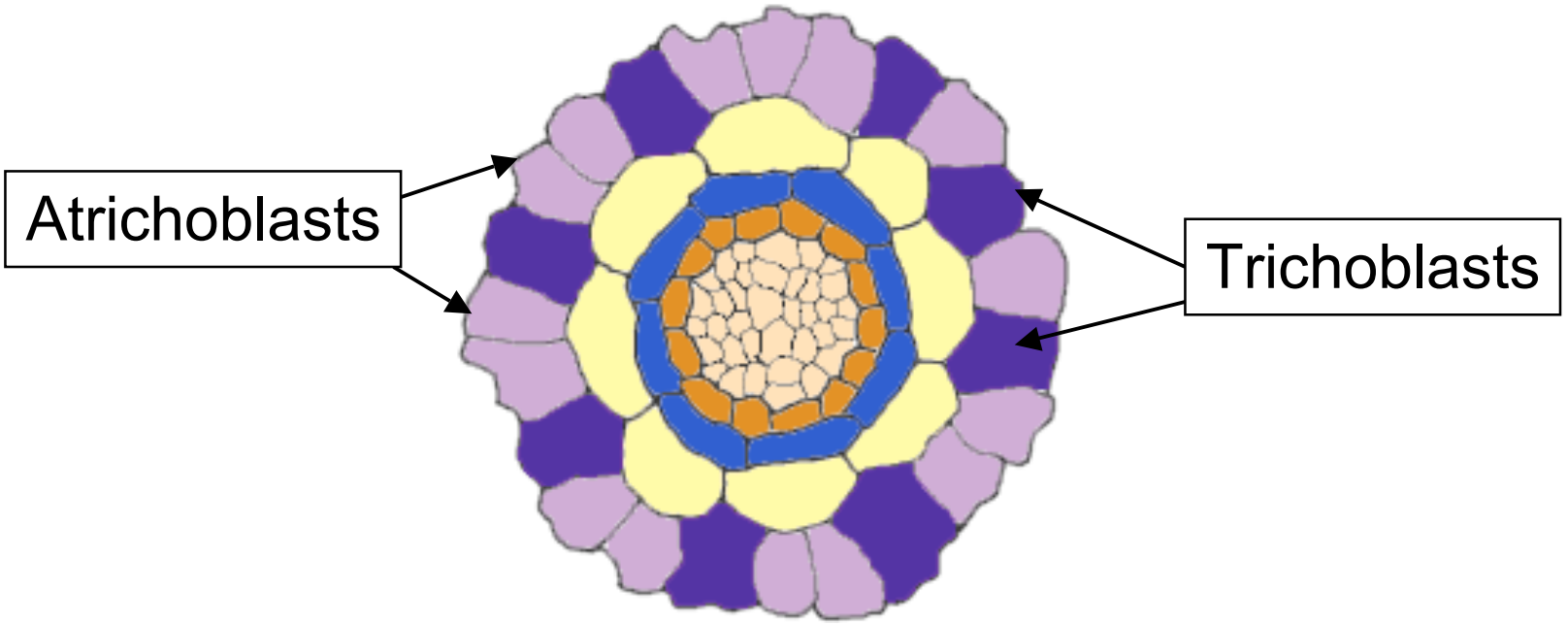
Multiple Different Cell Types Comprise the Root:



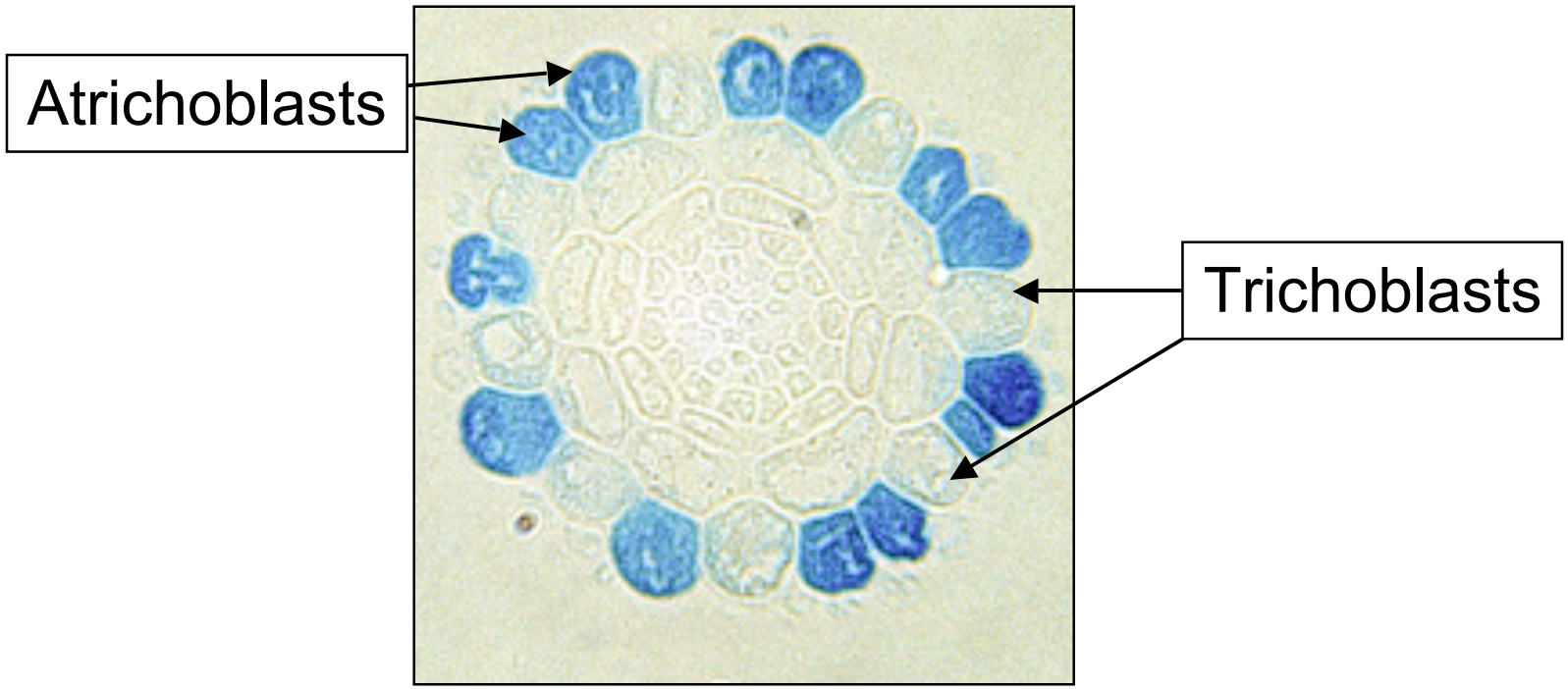
The Arabidopsis Root:



The Arabidopsis Root:



Use of *GLABRA2* as a marker of cell fate



Claire Grierson and John Schiefelbein

In wild-type roots not all H position cells will develop as root hairs

GL2+ not a hair
GL2- hair

Wild-type (5 days)

In the H position

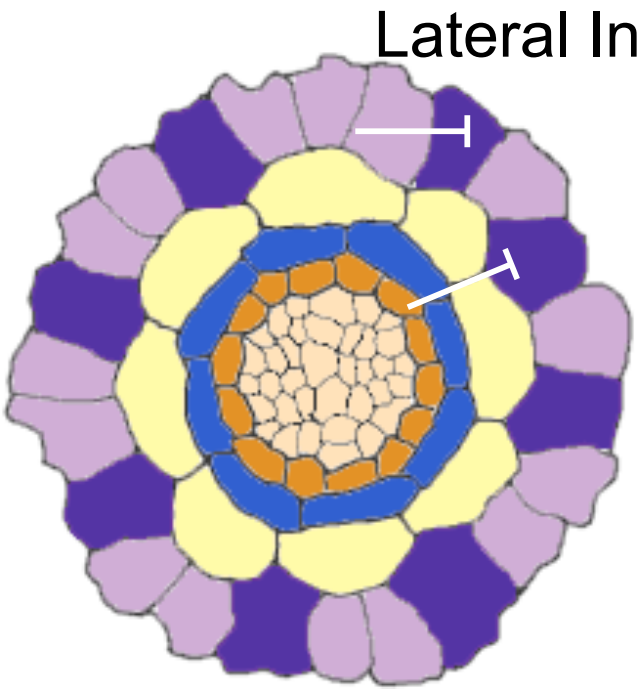
88.6±5.2 lack *GL2* expression
11.4±5.2 have *GL2* expression

In the N position

0.6±0.5 lack *GL2* expression
99.4±0.5 have *GL2* expression

Kwak, S.H. and J. Schiefelbein, *The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis.* Dev Biol, 2007. **302**(1): p. 118-31.

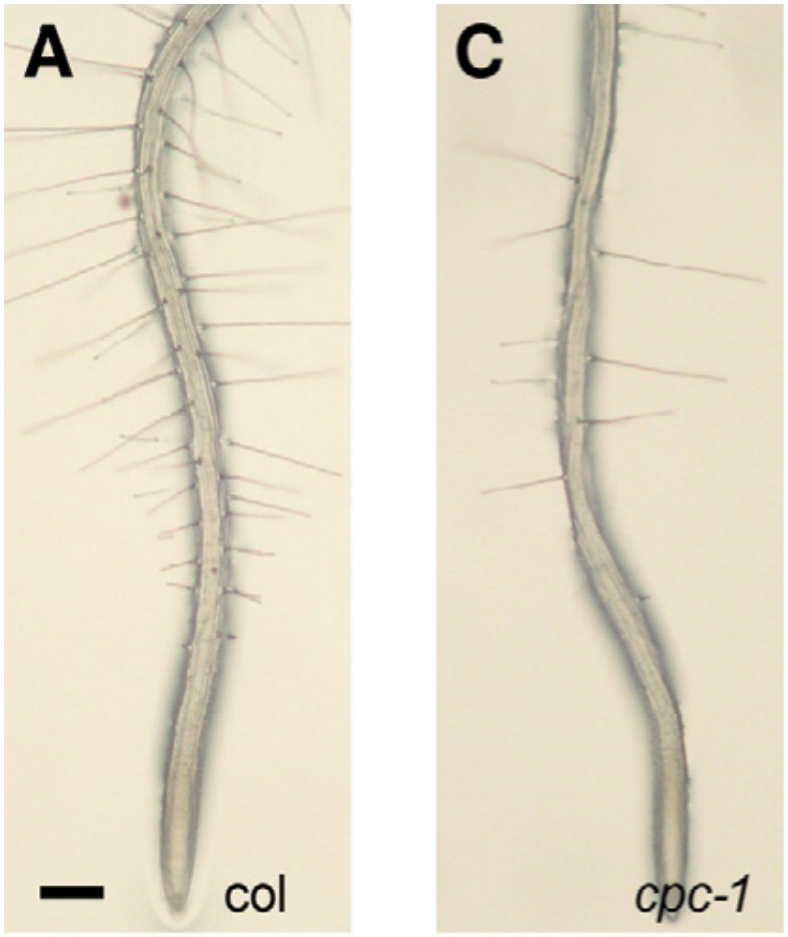
Patterning through lateral and radial inhibition of the N cell fate:



Wildtype plants have alternating rows of root hairs

Kwak, S.H. and J. Schiefelbein, *The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis.* Dev Biol, 2007. **302**(1): p. 118-31.

cpc *mutants:* make less root hairs than wt



Kwak, S.H. and J. Schiefelbein, *The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis.* Dev Biol, 2007. **302**(1): p. 118-31.

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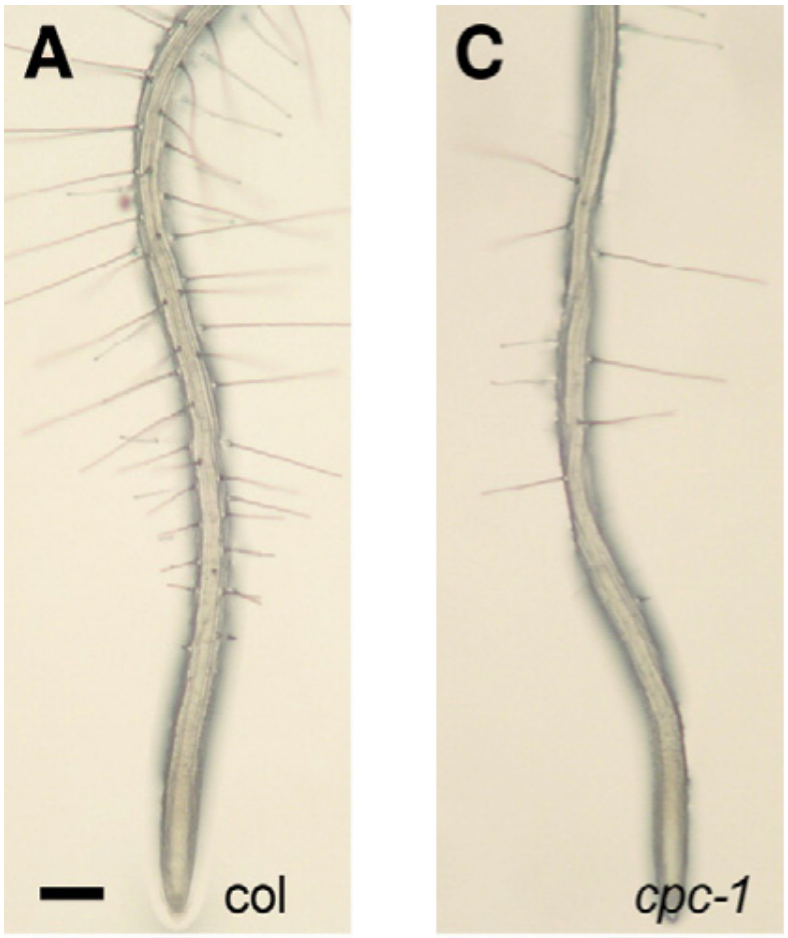
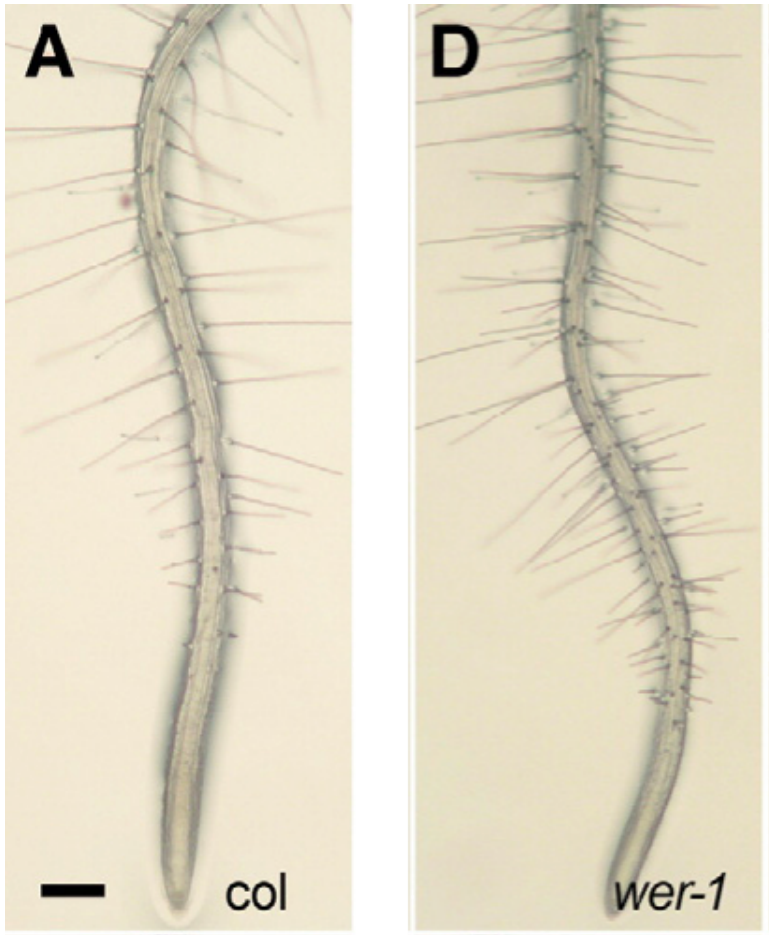


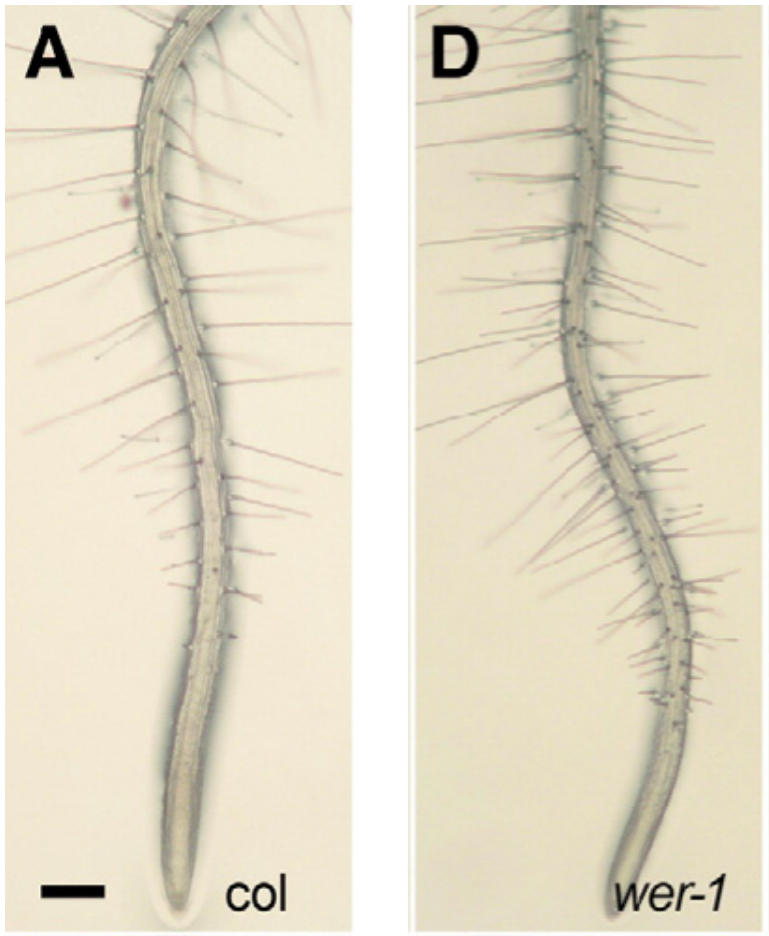
CPC is a positive regulator of root hair cell fate.

wer *mutants:* make more root hairs than wt



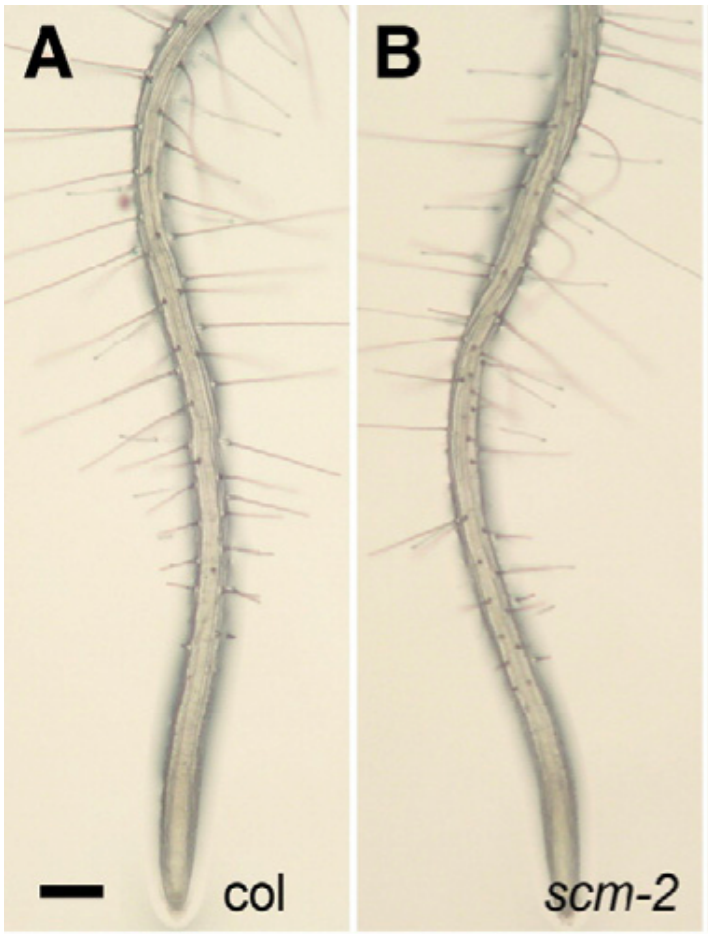
Kwak, S.H. and J. Schiefelbein, *The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis.* Dev Biol, 2007. **302**(1): p. 118-31.

wer *mutants:* make more root hairs than wt



WER is a negative regulator of root hair cell fate and a potential target of the lateral and radial signals.

scm mutants: make normal numbers of root hairs that are often inappropriately positioned.



Kwak, S.H. and J. Schiefelbein, *The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis.* Dev Biol, 2007. **302**(1): p. 118-31.

In *scm-2 mutants* there are hair cells in the non-hair cell position

GL2+ not a hair
GL2- hair

scm-2 (5 days)

In the H position

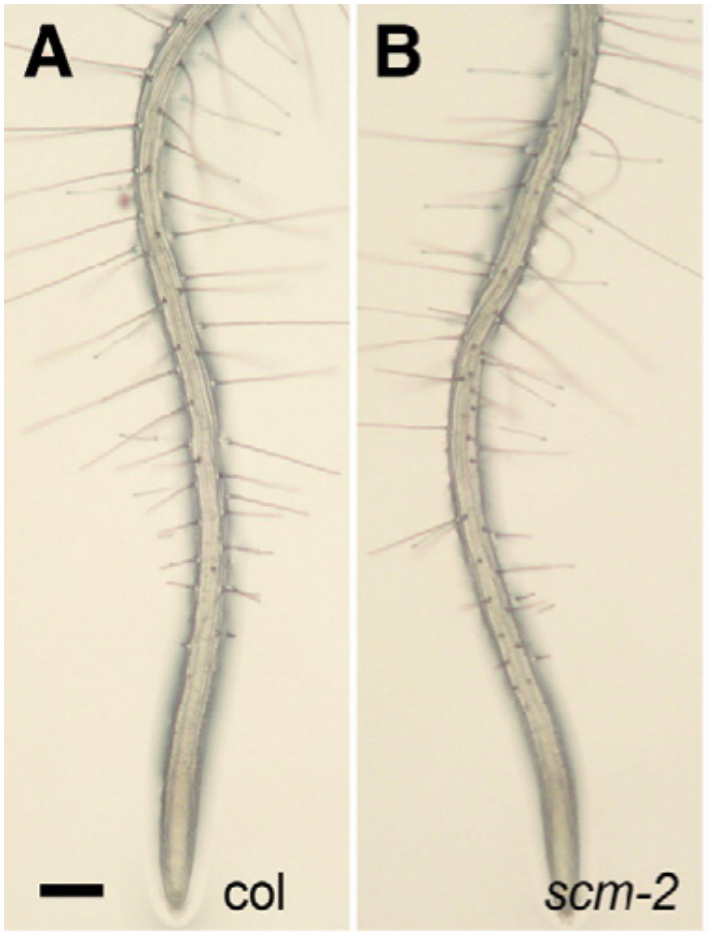
62.8±6.2 lack *GL2* expression
37.2±6.2 have *GL2* expression

In the N position

20.2±5.1 lack *GL2* expression
79.8±5.1have *GL2* expression

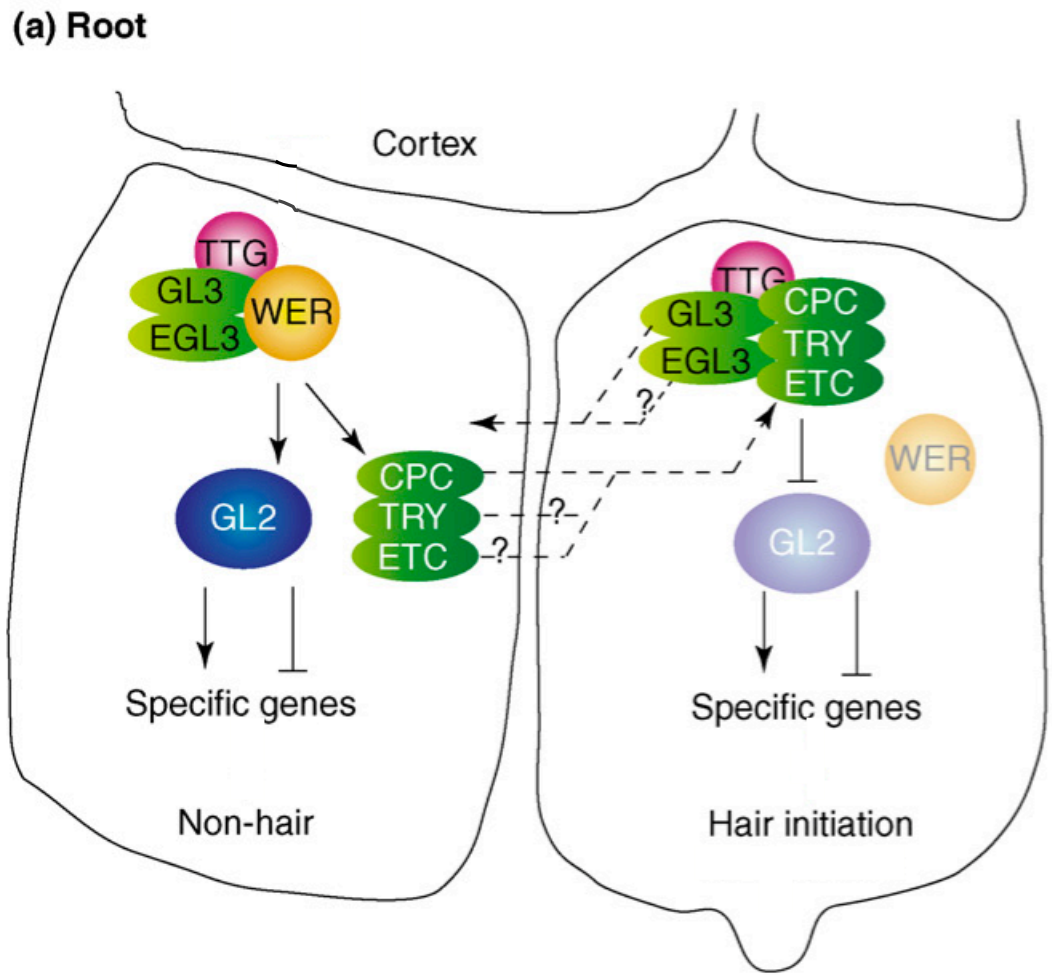
Kwak, S.H. and J. Schiefelbein, *The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis.* Dev Biol, 2007. **302**(1): p. 118-31.

scm mutants: make normal numbers of root hairs that are often inappropriately positioned.



Part of the lateral or radial signaling pathway.

Model for root hair patterning

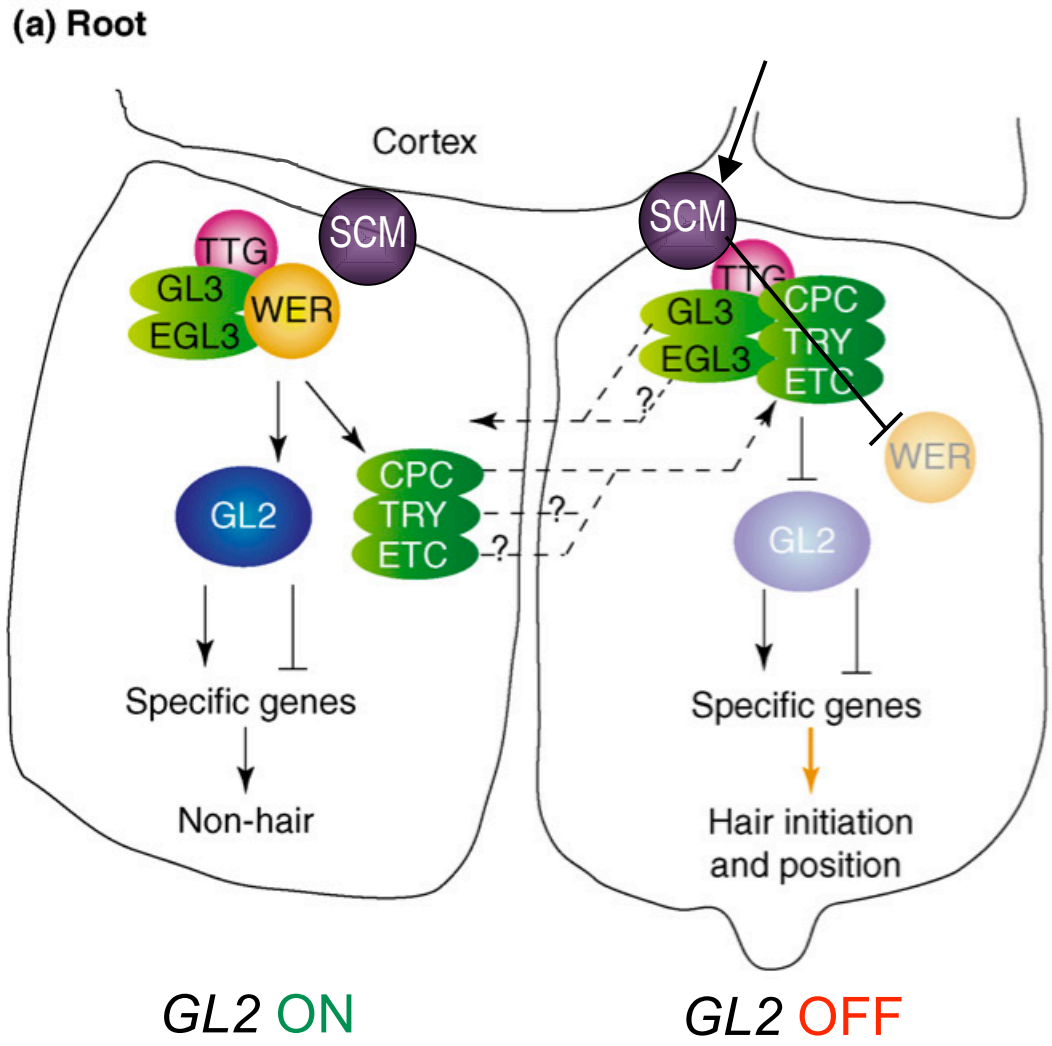


Modified from: Guimil, S. and C. Dunand, *Cell growth and differentiation in Arabidopsis epidermal cells.* J Exp Bot, 2007. 58(14): p. 3829-40.

GL2 ON

GL2 OFF

Model for root hair patterning



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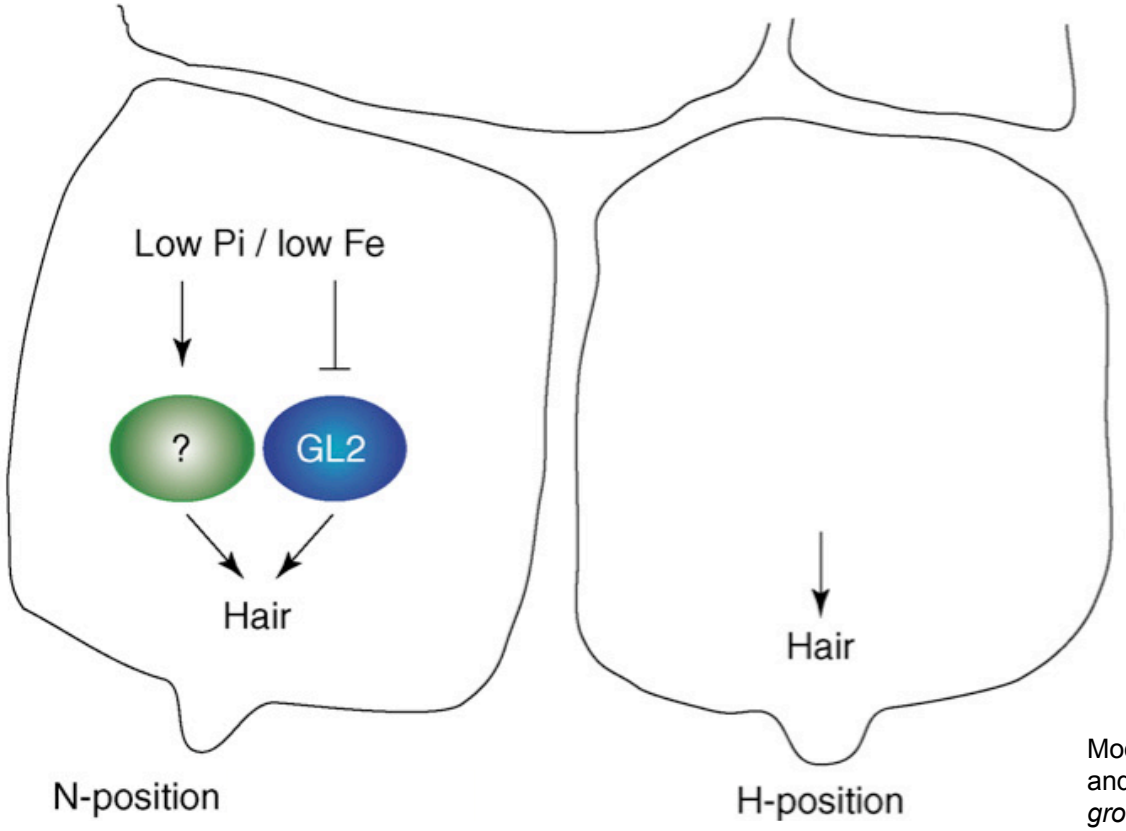
Affect of Nutrients on Root Hair Formation



Modified from: Guimil, S. and C. Dunand, *Cell growth and differentiation in Arabidopsis epidermal cells.* J Exp Bot, 2007. 58(14): p. 3829-40.

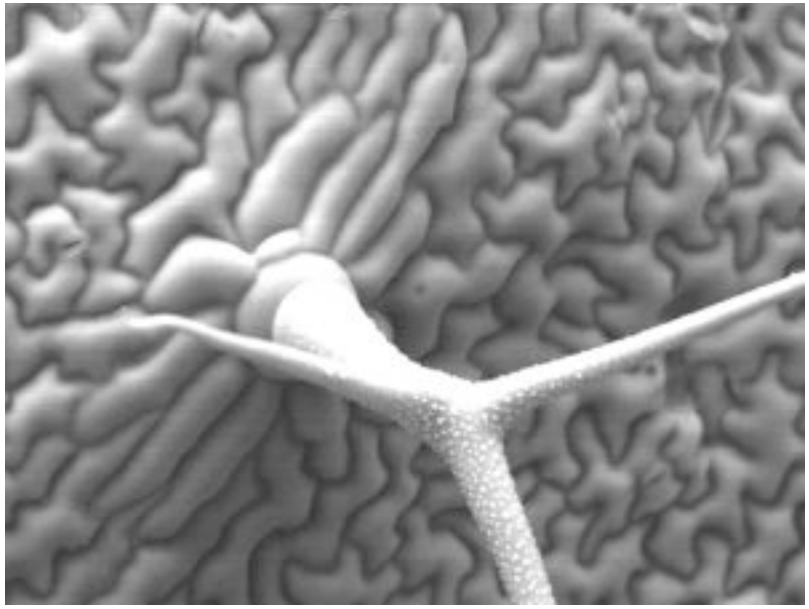
Ideas for expanding the lab:

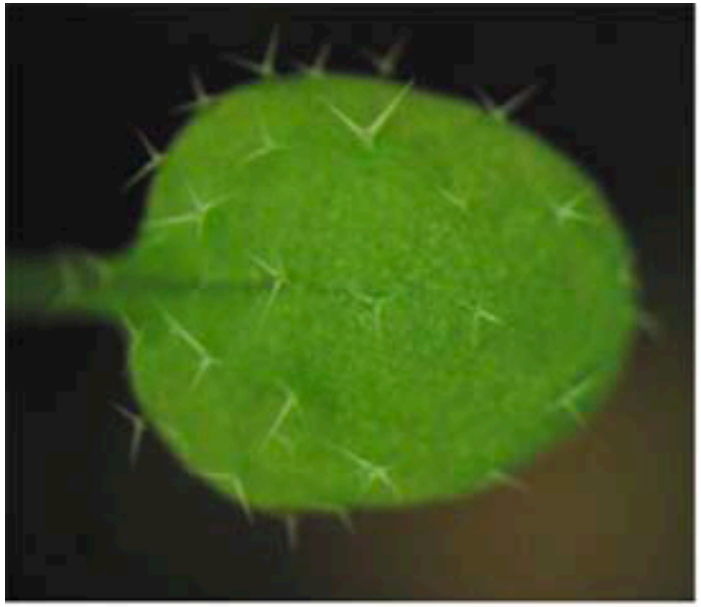
- Hormones and Root Hair Patterning (**auxin**: Knox, *et al*. Development 130, 5769-5777; **auxin and ethylene**: Rahman *et al*. *Plant Physiology, December 2002, Vol. 130, pp. 1908-1917*)

- Microarray Analysis (Dinneny *et al*. Science, 2008. **320**(5878): p. 942-5**.),**

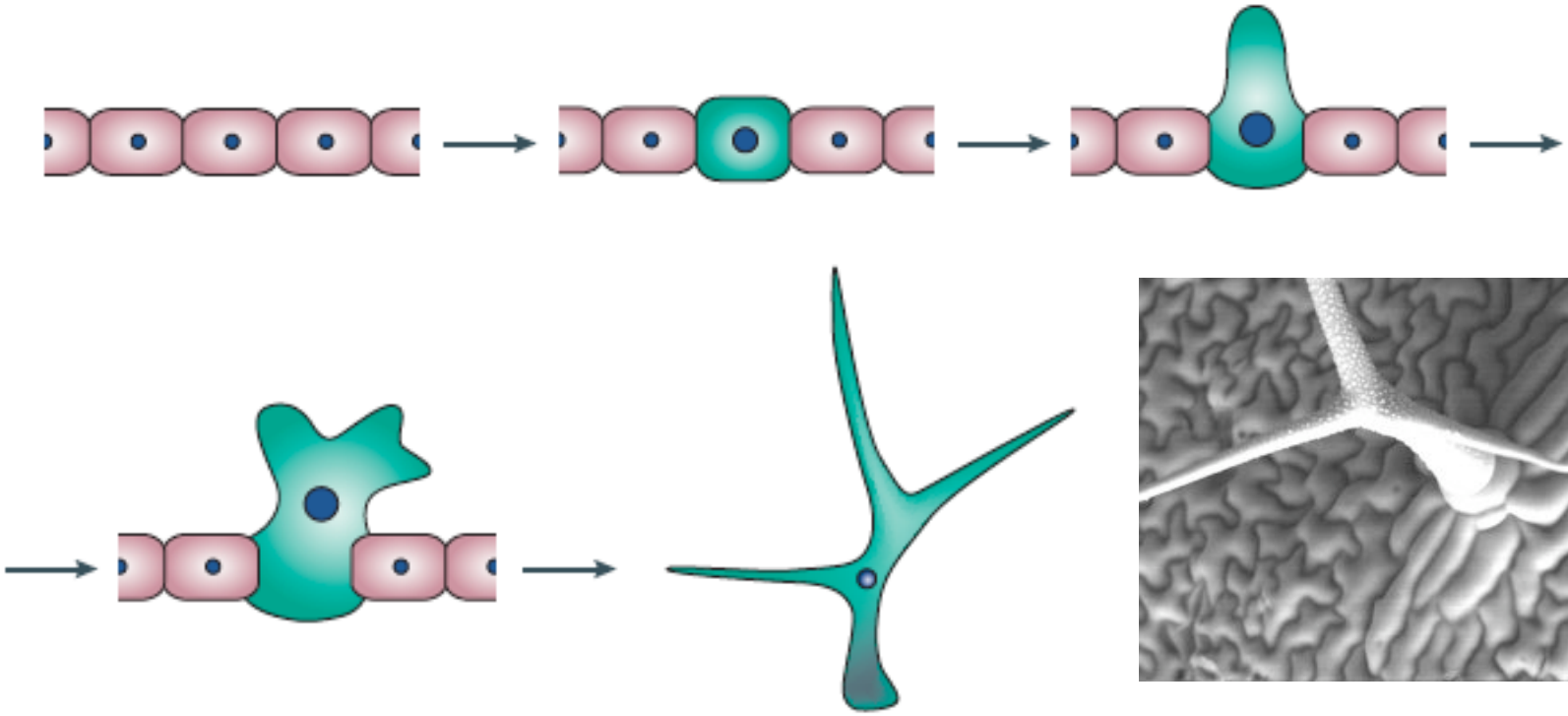
- Trichome formation

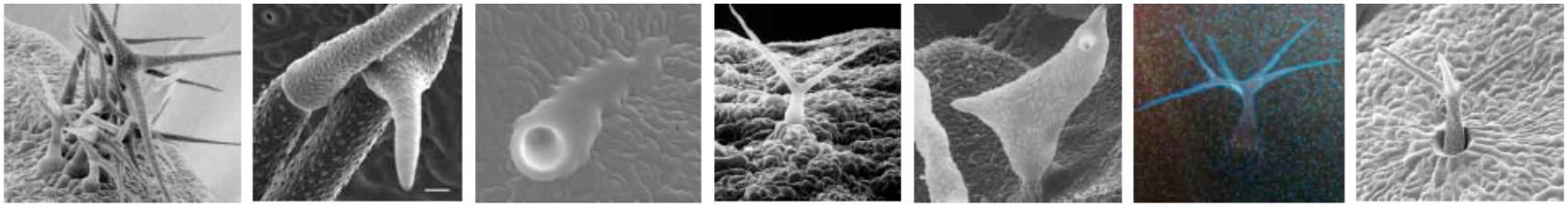
Trichomes



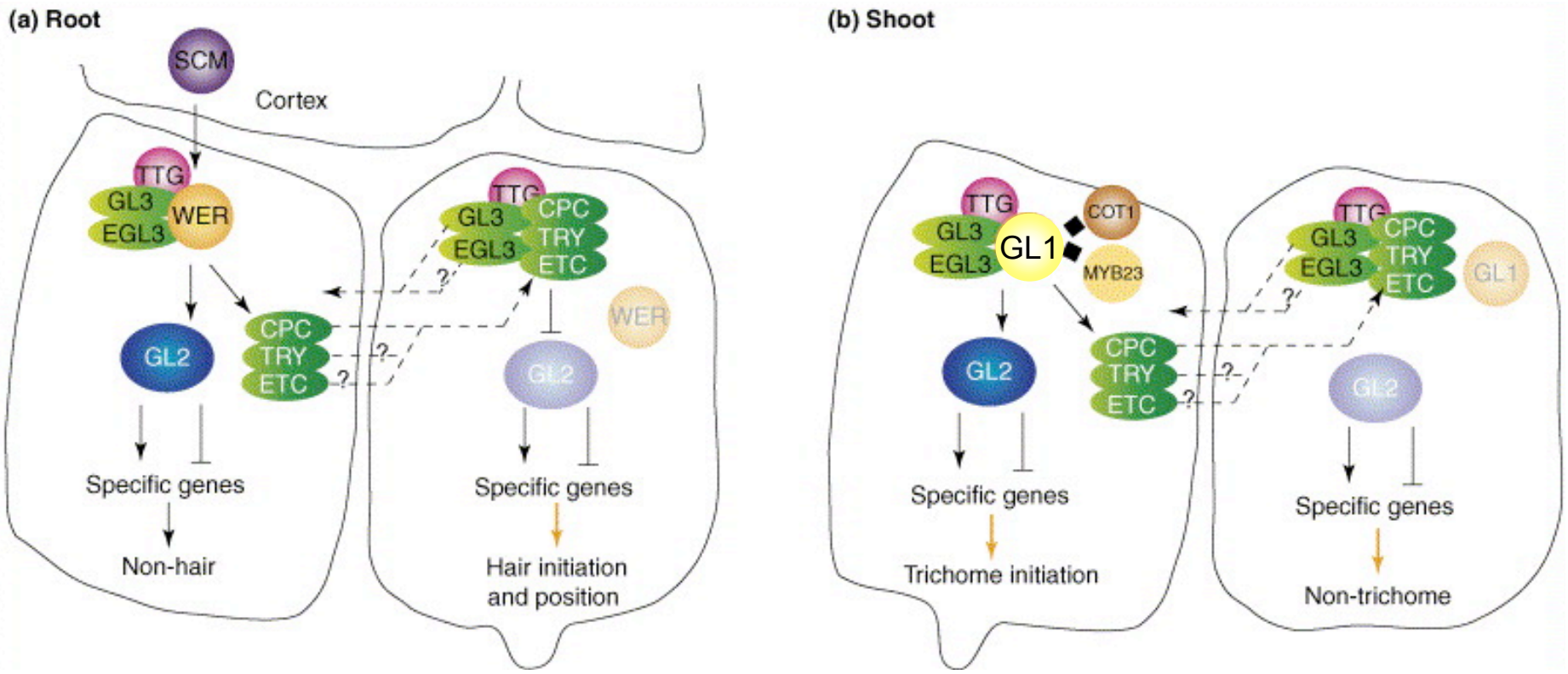


Trichome development



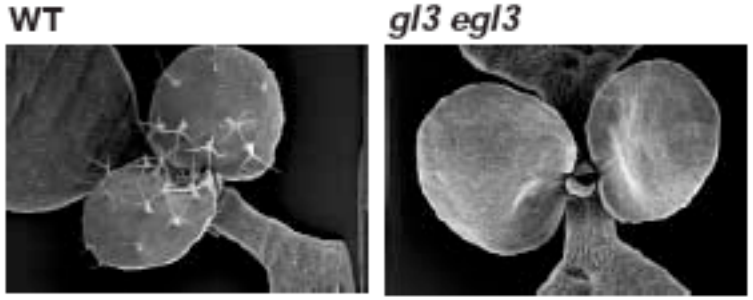


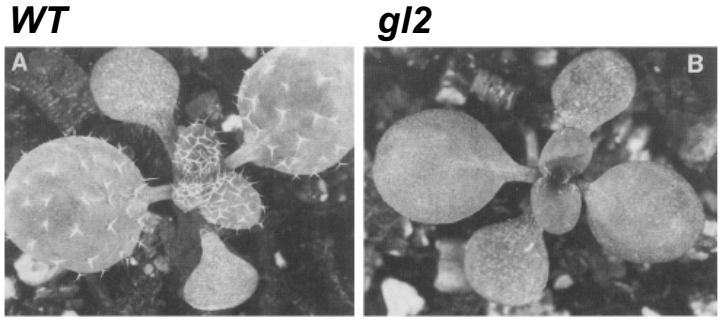
Similarities between root hair and trichome patterning

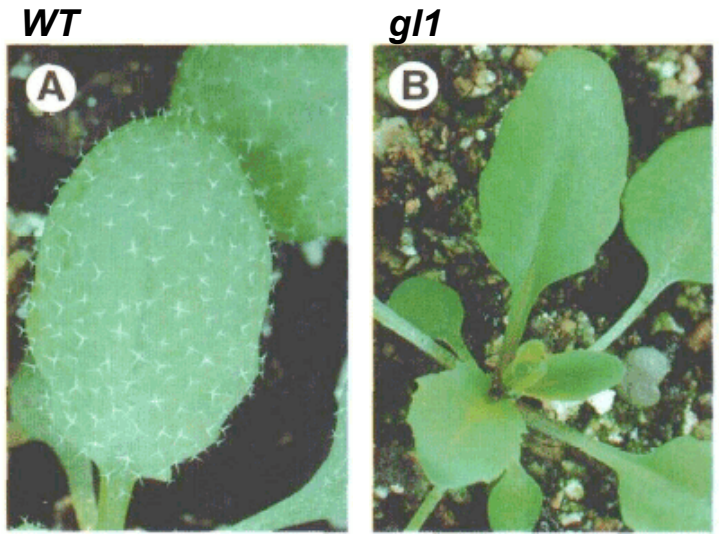


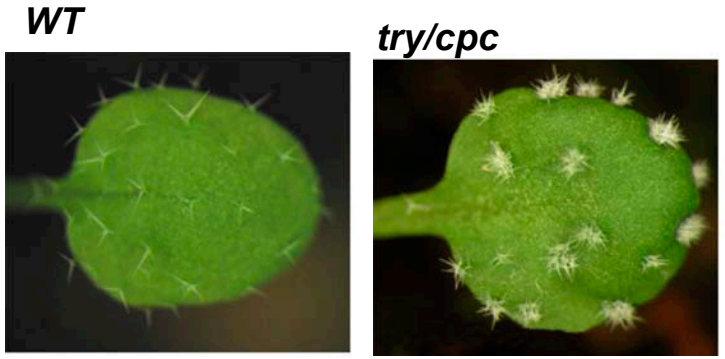
GL2 ON *GL2* OFF *GL2* ON *GL2* OFF

Mutations affecting trichome patterning









Acknowledgements

Steve Clark

John Schiefelbein

Scott Poethig

Terri Long

The *Arabidopsis* Community

This Document is from Steve Clark. I have added some notes and alternative suggestions in italics throughout.

Growth conditions

You can grow Arabidopsis in the greenhouse. You just have to be careful with temperature, as Arabidopsis does not do well at higher temperatures (20-22 C is ideal). Plant growth chambers provide a nice, constant environment. They can be expensive, and limit the amount of plants you can grow at any one time. There are many different manufacturers. Conviron growth chambers (www.conviron.com/) are popular in my Dept.

An easy and inexpensive way to set up growing conditions is to build your our plant growth rack. You can mount light fixtures to the undersides of metal shelving to provide a lot of growth space in a unit that will take up very little room. All of our growth is on chrome wire racks ordered from Global Industrial Supply (www.globalindustrial.com/) If you search under chrome wire shelving, you will find units like this:
http://www.globalindustrial.com/gcs/product/categoryInfo.web?options.parentCategoryKey=167&catSearchParams.categoryKey=1931&REQ_SUB_CAT=Chrome%20Wire%20Shelving&index=48

Our shelves are 5 foot x 2 foot, under which we mount four 2-bulb 48 inch fluorescent fixtures (for a total of 8 bulbs). You can get away with less lighting per shelf. It is important to buy electronic ballasts for the fluorescent fixtures to cut down on the amount of heat generated. It is also important to wire together the fixtures in such a way that if one bulb goes out, the other fixtures stay on. Using a shop to do this is a big help. You can go even smaller and cheaper than this by buying a pre-built rack from the hardware store and stringing a couple of fixtures underneath a shelf or two.

The home-made plant growth racks can be placed in any lab room, as long as it is air conditioned. Just be aware of the heat load the growth rack is adding to the room, and keep them as spread apart as necessary to stay reasonably cool.

Cool white fluorescent bulbs are fine (make sure to buy fixtures for and use the thinner, more energy efficient bulbs – the older, thicker bulbs are harder to find now). We never saw any difference with the "grow lights" you can buy from gardening stores, but they may work for you, especially if you have fewer bulbs per shelf. Incandescent lights are unnecessary, and, in fact, generate a lot of heat.

Put your plants on at least 16 hours of light per day. Short "photoperiods" (i.e., amount of light per 24 hour period) significantly delay flowering. Continuous light works fine.

Soil

Nearly every lab has a different preferred approach to planting and growing Arabidopsis. If you are growing only occasionally, I would recommend using a planting mix, like a SunGro Sunshine Mix (Mix #5 is popular). If you are growing the plants more continuously, consider trying a dryer mix to cut down on fungus and fungus gnats (see pests below). We use a 1:1:1 mix of vermiculite:perlite:Scotts Metromix 360. This makes for a much lighter mixture with less organic material.

Try to avoid using topsoil, as it can be very inconsistent. We used it is the past but stopped after getting some very bad batches that stunted plant growth.

Fertilizer

Any sort of fertilizer is fine. For best plant growth, at least some is helpful. Some people like to use fertilizer every time they water. We prefer to add a set amount of time-release fertilizer to each pot. We use half an microfuge tube (~1 ml) of Scotts Osmocote pellets (available at any hardware store) in each 3.5"x3.5" pot when doing the original planting.

Pots

Gage Industries, Inc.
P.O. Box 1318-6710 McEwan Road
Lake Oswego, OR 97035

http://www.gageindustries.com/
Catalog # GDP-350
1500 disposable pots

Trays

Carolina Biological Supply (www.carolina.com)
They have a variety of sizes. The catalog numbers are:

66-5910 (4 x 8 inches)	66-5912 (8 x 8)	66-5914 (8 x 12)
66-5916 (22 x 11)	66-5918 (13 x 15)	

Use whatever size suits your growing conditions (we use the 22 x 11).

Planting seeds

As with most other things, there are many ways to plant seeds, and everyone has their own preference. One thing you need is a cold imbibition for uniform germination. You can plant to seeds in pots that are thoroughly wet, and then place the pots at 4 C for four to seven days. Alternatively, you can place a wet piece of Whatman paper in a petri dish, add seeds, and place that at 4 C for four to seven days. This takes up much less space, but the wet seeds on the filter paper are harder to plant in soil.

Assuming you are planting dry seeds, you can use many different methods. One is to directly place seeds using wet forceps to pick up seeds (they will stick to the wet surface of the forceps – you do not need to grab the seeds by squeezing the tips together) and transferring them one at a time to the pot. In a 3.5 x3.5 pot, we place 16 seeds for optimal growth. More than that and the plants start to suffer from crowding. You can place the seeds on a piece of paper and sprinkle them onto the soil. This is much faster, but gives rise to clumping. Others like to place seeds in a water of a dilute agarose solution and pipet them onto the soild. For Whatman-imbibed seeds, you can grab a chunk of the filter paper with forceps and get the attached seeds and transfer this to soil.

Cover your trays in Saran Wrap and place them under the lights. Leave space at the edges for air flow, which is important for water evaporation for cooling. The wrap can be removed after 3-4 days. Keep standing water in the trays during germination, but try not to do so during normal growth.

Seeds storage

Arabidopsis seeds are stable for many years if stored properly. The first step is to collect seeds from healthy plants. Sickly plants do not give rise to happy seeds. You can collect the seeds by rubbing the dry fruits (after they have turned brown) over a piece of paper. The "chaff" can be separated from the seeds either by passing through cheesecloth or by simply brushing the chaff off with your fingers. *We use sieves that are usually available in any Chinatown market. We find this much easier than the cheesecloth or brushing methods. For picture: http://www.cookingforengineers.com/pics/640/DSC_1703_crop.jpg*

Once collected, seeds can be stored either in a microfuge tube or small envelope. You can special order very small envelops from your local office supply store. Either way, seeds stay best if they are dry, and that can be achieved by place the tube rack or envelops into tupperware with a layer of drierite. Seeds do best at 4 C, but room temperature storage is fine.

Mutant lines and general information

The TAIR web site is the most important (http://www.arabidopsis.org/). This is a collection of information and search tools for Arabidopsis. Stocks are maintained by the ABRC at Ohio State, with links on the TAIR homepage for stocks and ordering.

Knock-out lines

These are ordered through the ABRC, but the best search engine for knock-outs in particular genes is at the Salk Signal database (http://signal.salk.edu/). If you follow the link for "T-DNA Express" on the Salk Signal homepage, you can search every section of the genome for ESTs and knock-out lines. All you need is DNA sequence or a gene name. *The T-DNA express link:* http://signal.salk.edu/cgi-bin/tdnaexpress

Arabidopsis nomenclature

While not every gene adheres to the Arabidopsis nomenclature rules, the vast majority do. Each gene is given a unique designation of up to 3 letters and a number (e.g., *CLV1* for the gene *CLAVATA1*). Wild-type alleles are written in all capital letters in italics. Mutant alleles are written in lower case in italics (e.g., *clv1*). Different mutant alleles are distinguished by an allele number, that are generally designated in order (e.g., *clv1-1*, *clv1-2*, *clv1-3*, etc.). In competitive fields, researchers will sometimes jump far ahead in allele numbers in order to avoid duplicate allele names while keeping their research secret until publishing. For example, there are *BRASSINOSTEROID1* (*BRI1*) alleles *bri1-101*, *bri1-201*, *bri1-301*, even though there are not 300 different *bri1* alleles. Dominant alleles are sometimes, but not always, followed by a "d" (e.g., *phb-1d*). *Proteins are indicated by capital letters and are not italicized (*CLV1 *is the CLV1 protein). The Arabidopsis phenotype is indicated by italics with the first letter capitalized so Clv is the phenotype. Most people do not use this convention and instead use clv11 to indicate a specific phenotype.*

Pests

Growing Arabidopsis means growing pests as well. Pests are much more of a problem for continuous growth than occasional plantings, as a constant source of plants can sustain a high pest population. If you are simply growing plants every once in a while for a lab demonstration, pests will be at most a minor problem. The following advice below is mainly for continually grown *Arabidopsis*.

Preventing contamination: Try not to go to plant growth rooms or chambers immediately after entering the building. You can pick up pests on your clothing and bring them inside. Try not to go directly from one plant growth space or chamber to another, as this can spread contamination. If you have two spaces or chambers in which you can grow plants, try to rotate plantings between them. Finally, remove plants as soon as they are dead, or as soon as you are finished with them. Some pests require weeks to complete their life cycle, and removing material quickly can disrupt this.

Common Pests:

Fungus gnats: These are small, black, flying gnats. The adults are harmless, but the larvae live in the soil and eat the roots and leaves of the plants. A heavily infested pot can be devoured quite rapidly. *Fungal gnats are often the result of over watering your plants. If possible water from the top and allow the soil to dry out between waterings. There are multiple species of predatory nematodes (like Steinemema carpocapsae & Heterorhabditis heliothedis) that can be used to help keep the fungal gnat larvae at bay.*

1. Control: Fungus gnats are the hardest of the insects to completely eliminate since the larvae live in the soil and are less accessible with pesticides. Chemical control can involve regular "bombing" to intense spraying. For continuous growth rooms, we will use a house-hold bug fogger every other week. We turn off the lights and ventilation, set off the fogger, and leave until morning. Intense spraying can involve both Orthene and Malathion. Both are available at your local hardware store. **Non-chemical treatments involve both the sticky flytraps and Gnatrol** (*this is the method that we use at Penn. We apply the Gnatrol to the soil when planting*). Place the flytraps on level with you plant trays, and the adult gnats will fly into them and get stuck. Gnatrol is added to the soil by mixing with the water used to initially wet the soild and/or later when watering the pots. It contains a bacterial solution that secrete a compound toxic to the larvae. Gnatrol is a bit smelly and messy, but it will reduce the problem. Gnatrol is available many places and can even be bought from Amazon.com.

2. Aphids: Aphids are small, light green sucking insects. They generally attach themselves to the stem and do not move around, although they can be found on the underside of leaves as well. They reproduce relatively quickly and can decimate the plants. Aphids are the easiest pest to completely eliminate. Spraying orthene on all of the plants in a growth area every 3 days for 2 weeks is usually good enough to eliminate them. *At Penn and at Duke, we've used ladybugs with reasonable success. Once the aphids are gone however, the ladybugs will also die, so you need to replenish your supply.*

3. Thrips: Thrips are the worst pest we have experienced. They are small, yellowish insects that can move pretty fast. They crawl into shoots and flowers and feed on the growing tissue, causing it to shrivel up. They are not as easy to spot as aphids or gnats, and can be very well spread across many plants before they are noticed. A sure sign of thrips are small black spots around the apices of plants, especially if the plants look sickly. The only thing we have found that controls thrips is orthene. Thrips are harder to completely eliminate than aphids. One reason is that they can lay eggs in the seeds. This can be treated by placing the DRY seeds (freshly collected recently mature seeds might not survive) overnight at –80 C. We normally do this now right before imbibing the seeds.