

Background

- Epidermis of *Arabidopsis* cotyledons is an excellent material for studying cell proliferation because the epidermal cells, including pavement cells, guard cells, and meristemoids (Fig. 1), are easily accessible and cell divisions cease at an early developmental stage.
- The Landsberg *erecta* (Ler-0) accession of *Arabidopsis* has more pavement cells, guard cells, and meristemoids than Columbia-0 (Col-0) in the cotyledon (Yang, 2016).
- Identification of the genes responsible for the epidermal cell number differences between Ler-0 and Col-0 will likely advance the understanding of the molecular mechanism regulating cell proliferation in plant organs.

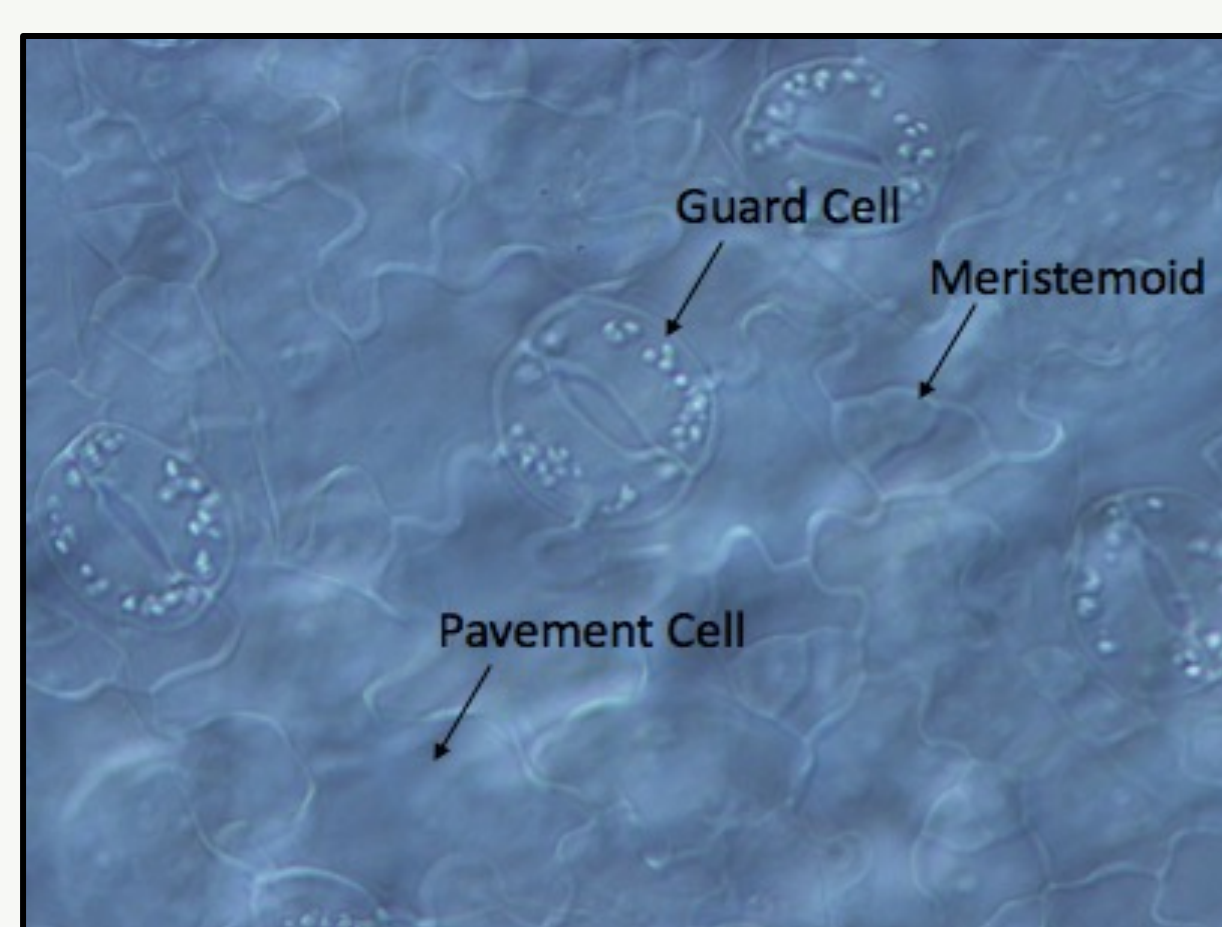


Fig. 1. Epidermal cells in an Ler-0 cotyledon.

Objective

To determine which genes are responsible for the differences in the epidermal cell numbers between Col-0 and Ler-0.

Methods

- Analyzed F₁ and F₂ plants from the crosses between Col-0 and Ler-0.
- Determined cell densities, cotyledon areas, and cell numbers to characterize phenotypes of F₁ and F₂ individuals.
- Extracted DNA samples from F₂ individuals with the Ler-0 epidermal phenotype.
- Mapped a gene of interest using these DNA samples and InDel polymorphic primers (Fig. 2).
- Harvested F₃ seeds from individuals of the mapping population for confirming their F₂ phenotyping results if needed.



Fig. 2. Example for mapping a PCR marker on chromosome 2 with F₂ individuals and controls.

Results

- Number & densities of pavement cells and meristemoids significantly differ between Ler-0 and Col-0 in mature cotyledons (Figs. 3 and 4; t-tests, $p < 0.001$, $n = 40$).
- Average cotyledon areas are not statistically different between Ler-0 and Col-0 (t-test, $p = 0.17$; $n = 40$).

Results

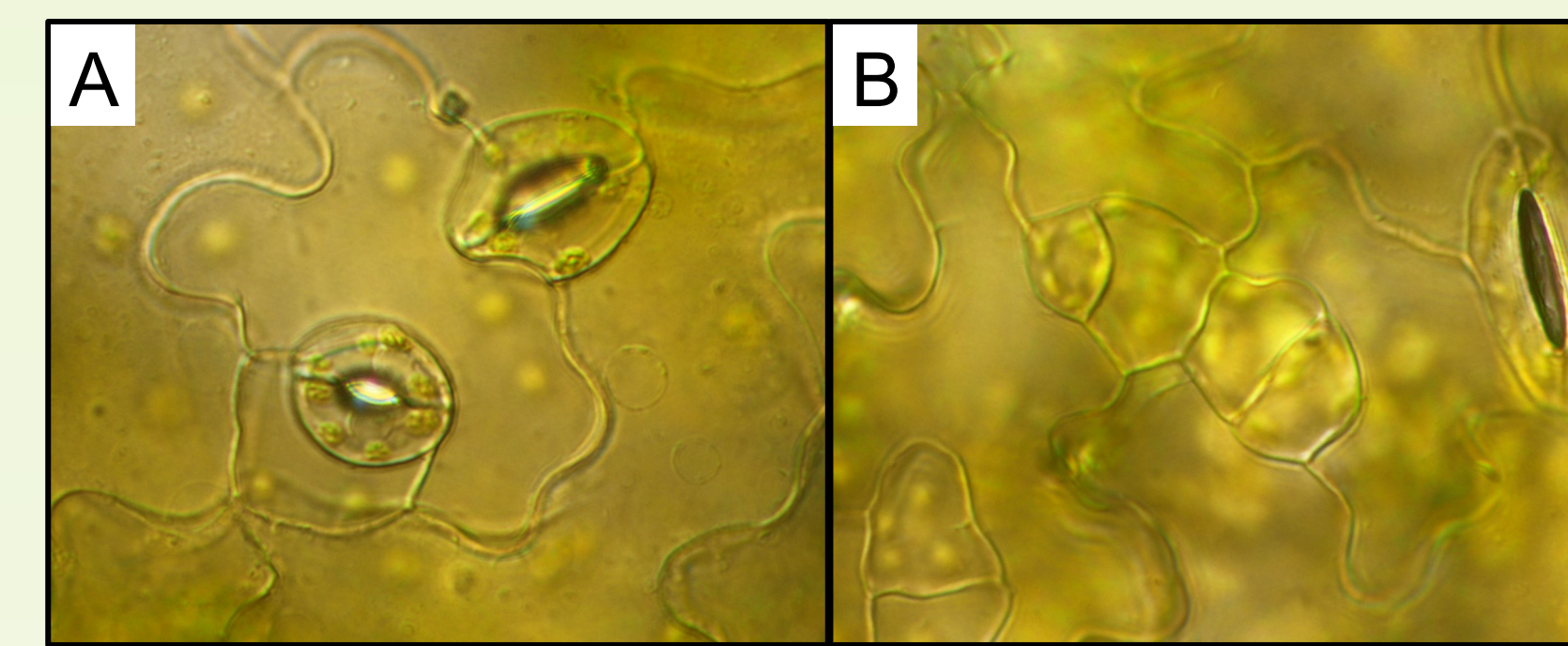


Fig. 3. Mature cotyledon abaxial epidermis, showing more meristemoids in Ler-0 than in Col-0. (A) Col-0. (B) Ler-0.

- F₁ plants showed an epidermal phenotype intermediate between those of Col-0 and Ler-0, indicating that the gene(s) in Ler-0 responsible for the epidermal phenotype is(are) semi-dominant (Fig. 4).
- The ratio of non-Ler-0-like plants to Ler-0-like plants in the F₂ was 7.2 : 1 (311 : 43), which rejects the hypotheses that one gene or two unlinked genes underlie the Ler-0-like phenotype, (χ^2 tests, $\chi^2 \geq 20.99$, $P < 0.005$).

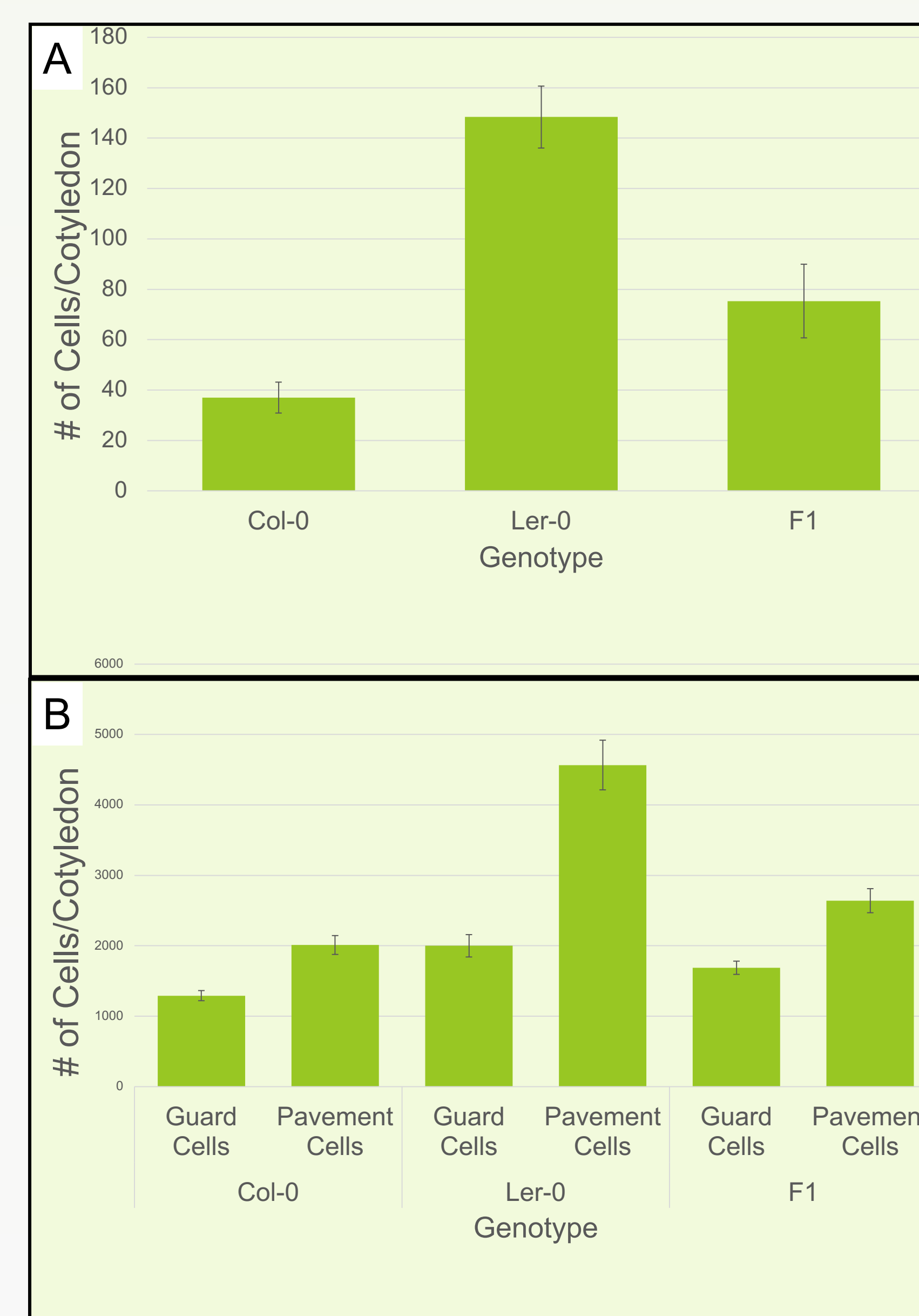


Fig. 4. Total cell number of Ler-0, Col-0, and F₁ mature cotyledons. (A) Meristemoids. (B) Pavement cells and guard Cells.

- A gene of interest is mapped to a ~900 kb region on chromosome 2 between At2G27130 & At2G29120 (Fig. 5).

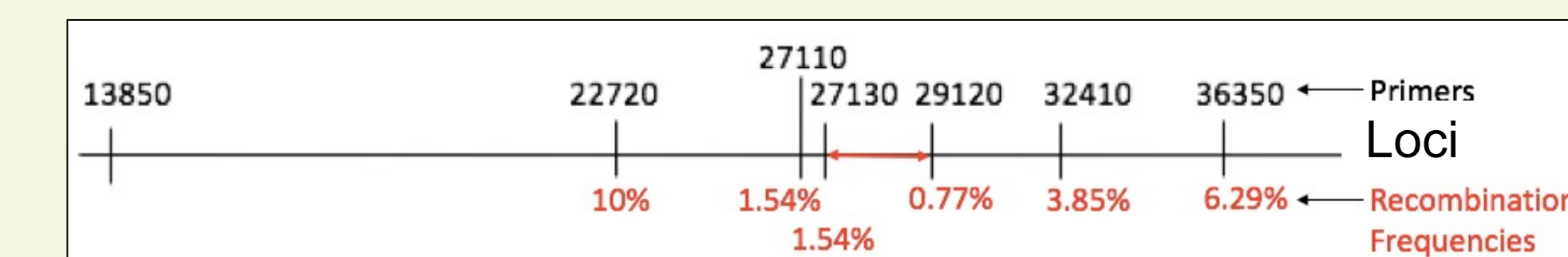


Fig. 5. Fine mapping of a gene of interest on Chromosome 2.

Discussion & Conclusions

- In the region between At2G27130 and At2G29120, there is no gene that is known to be involved in the regulation of epidermal cell production. Therefore, a novel gene is responsible for the Ler-0-like phenotype.
- According to the genetic analysis, two linked genes underlie the Ler-0-like epidermal phenotype.
- ERECTA* or At2G26330, the gene encoding a Leucine-rich repeat receptor kinase involved in the regulation of stomatal lineage formation, is close to the gene of interest, which is consistent with the above prediction.

Reference

Yang M. (2016) The FOUR LIPS (FLP) and MYB88 genes conditionally suppress the production of nonstomatal epidermal cells in *Arabidopsis* cotyledons. *Am J Bot*, 103: 1559-66.

Funding Sources:

American Society of Plant Biologists
Summer Undergraduate Research Fellowship

Oklahoma Center for the Advancement
of Science and Technology

Contact:
Jenny Swinton
Oklahoma State University
jenny.swinton@okstate.edu

