Novel Mutations That Affect Stomata Development in *Arabidopsis thaliana*

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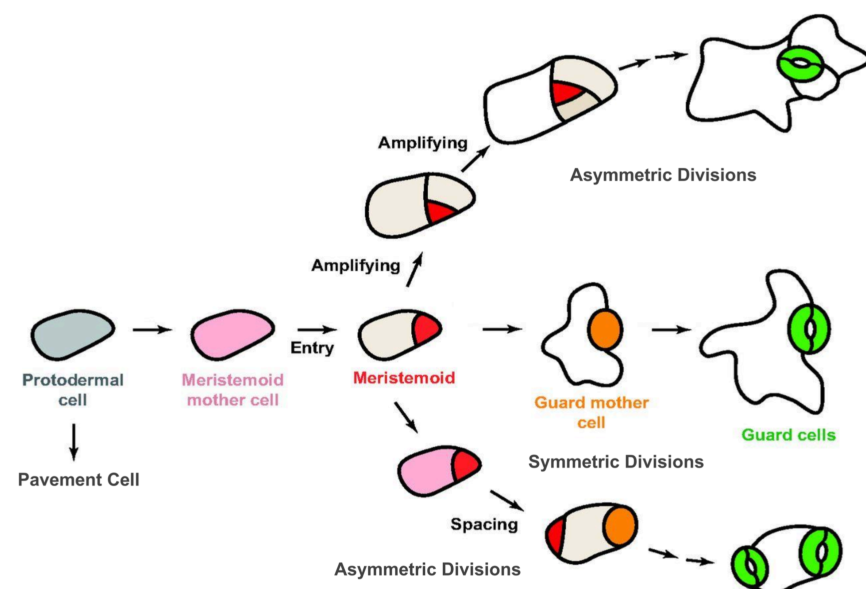
Abstract

Located on the epidermal surface of plants, stomata are small, pore-like structures that act as channels to exchange gas and water vapor between plant cells and the environment. Concentrations of gases and water within the plant cell are regulated through opening and closing of the stomata by turgor-driven movements. In *Arabidopsis thaliana*, development and differentiation of cells is controlled by the *ERECTA* (*ER*) family of genes (*ERECTA*, *ERL1*, and *ERL2*) which encode leucine-rich repeat-receptor-like kinases (LRR-RLKs). Acting synergistically, they direct cell division in different tissues and formation of stomata in epidermis. To better understand how ERECTA family genes regulate stomata development we conducted a forward genetic screen. Approximately 10,000 seeds of *erl1erl2* were mutagenized using ethyl methanesulfonate (EMS). The M_1 plants were grown and the M_2 seeds were collected. Then, M_2 seedlings were microscopically screened for stomata clustering. Two mutants, JMC_{19} and MC_{12} were chosen to pursue further because a high percentage of stomata in clusters was observed in their cotyledons. Both mutant lines were crossed with *erl1erl2* in order to rid their genomes of other EMS induced mutations and to determine the nature of obtained mutations (recessive versus dominant; single or double). The phenotype of novel mutants (stomata index and stomata clustering) was compared to the *erl1erl2*. The two lines were also crossed with Col to see if the phenotype of novel mutations depended on *erl1*and/or *erl2* mutations. After characterization of mutations, determining the location of the mutated JMC_{19} and MC_{12} genes through positional cloning is the next step. JMC_{19} and MC_{12} were crossed with Landsberg *erecta* (L*er*) to analyze recombination frequency between mutant phenotype and a set of genetic markers. The frequency at which the mutant gene(s) recombined with markers on L*er* chromosomes determined the location of the MC_{12}. This method will also be used for JMC_{19} in the future. The overall goal of the study is to understand, through the use of forward genetics, the mechanism by which stomata are spaced and to identify the gene(s) that control this developmental process.

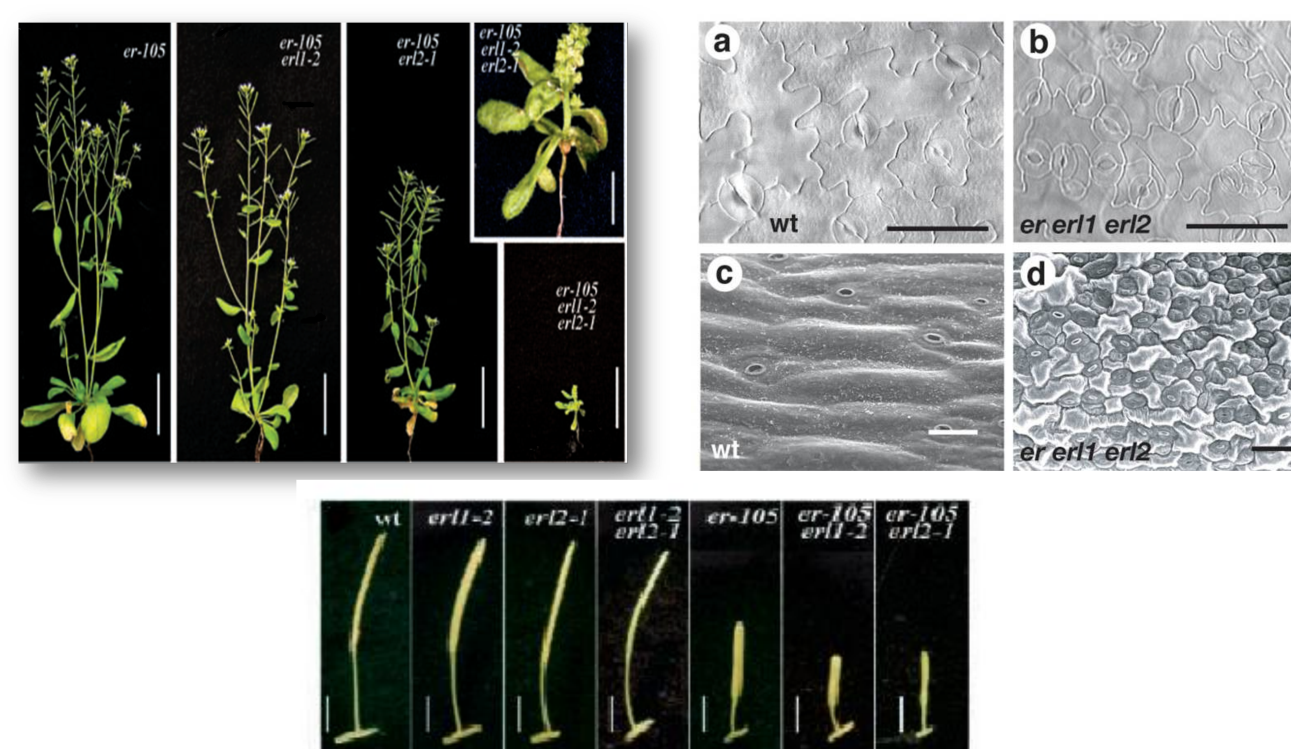
Background

Stomata Development



Stomata formation begins with a protodermal cell. This stem cell then can differentiate into a meristemoid mother cell (MMC) or a pavement cell. An MMC divides asymmetrically forming a meristemoid. Then, a guard mother cell (GMC) is formed from the meristemoid. Finally, the GMC yields two guard cells with an opening in the center; a stoma[1].

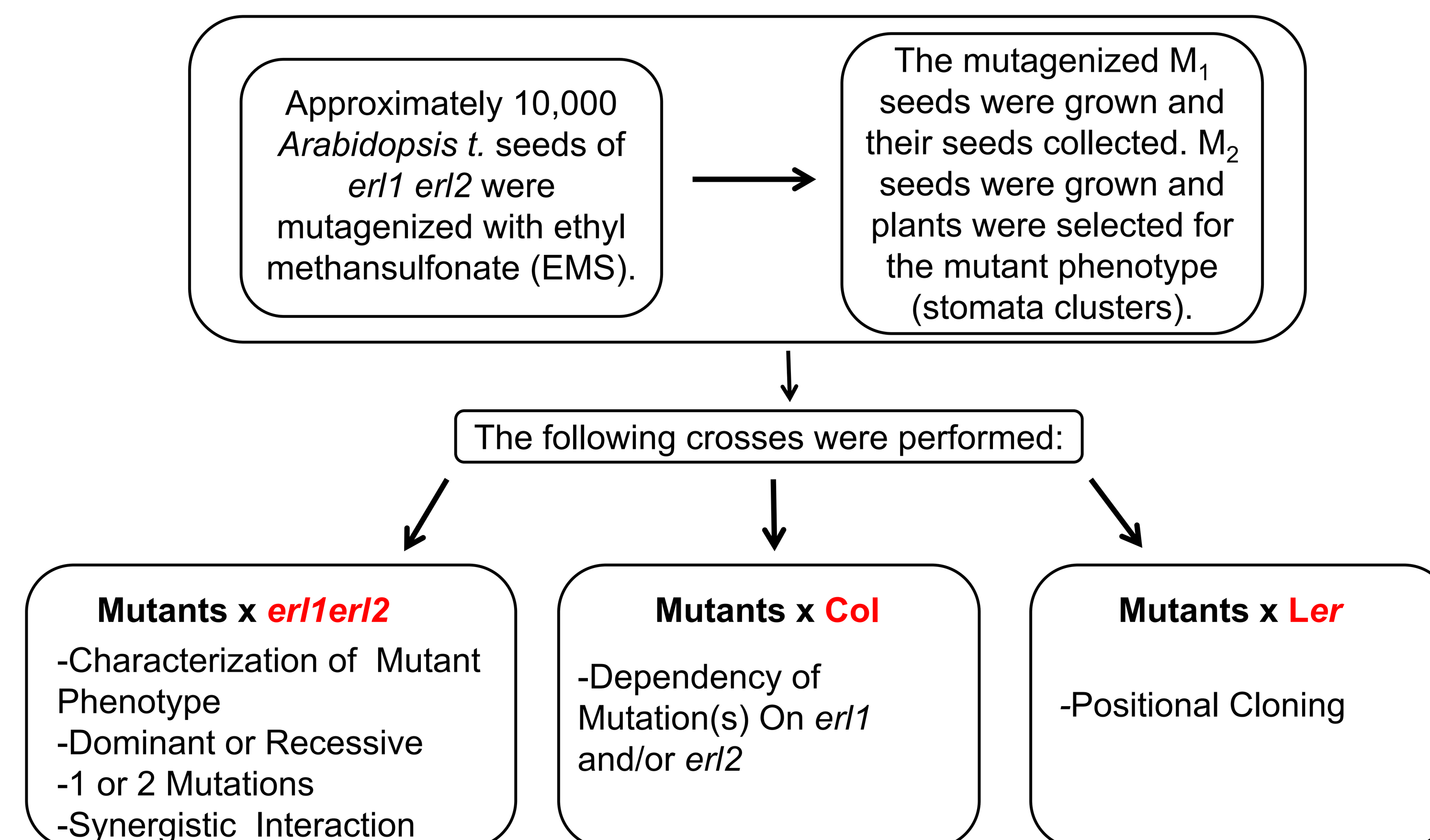
ERL1 and *ERL2* Act Synergistically With *ERECTA*



ERECTA (ER) family of genes (*ERECTA*, *ERL1*, and *ERL2*) synergistically promote organ development and growth. In comparison to WT, an *er* mutant has a phenotype of short height, short siliques, and compact inflorescence. When a mutant has either *erl1* and/or *erl2* mutations only, the expressed phenotype is drastically weaker. Double *er erl2* and er erl1 mutants have even stronger phenotypes and shorter in stature. When all three genes are mutated (*er erl1 erl2*), the plant is extremely short and compact. Over-proliferation of stomata and development of stomatal clusters is evident in the triple mutant[2].

Results

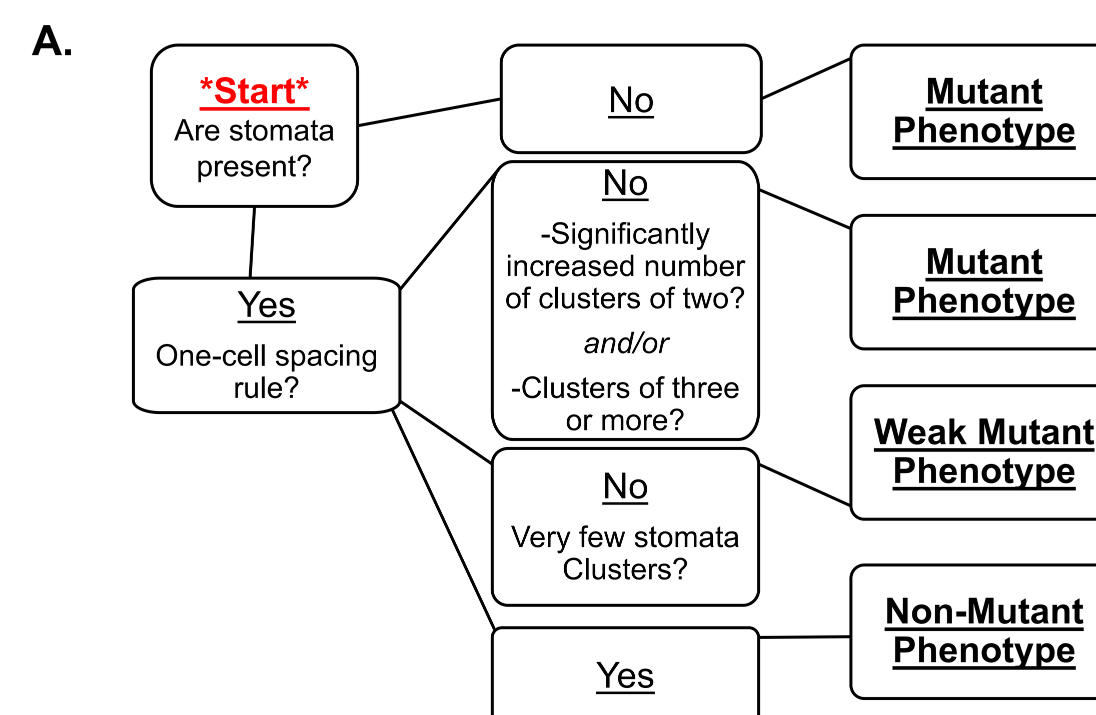
Enhancer Genetic Screen

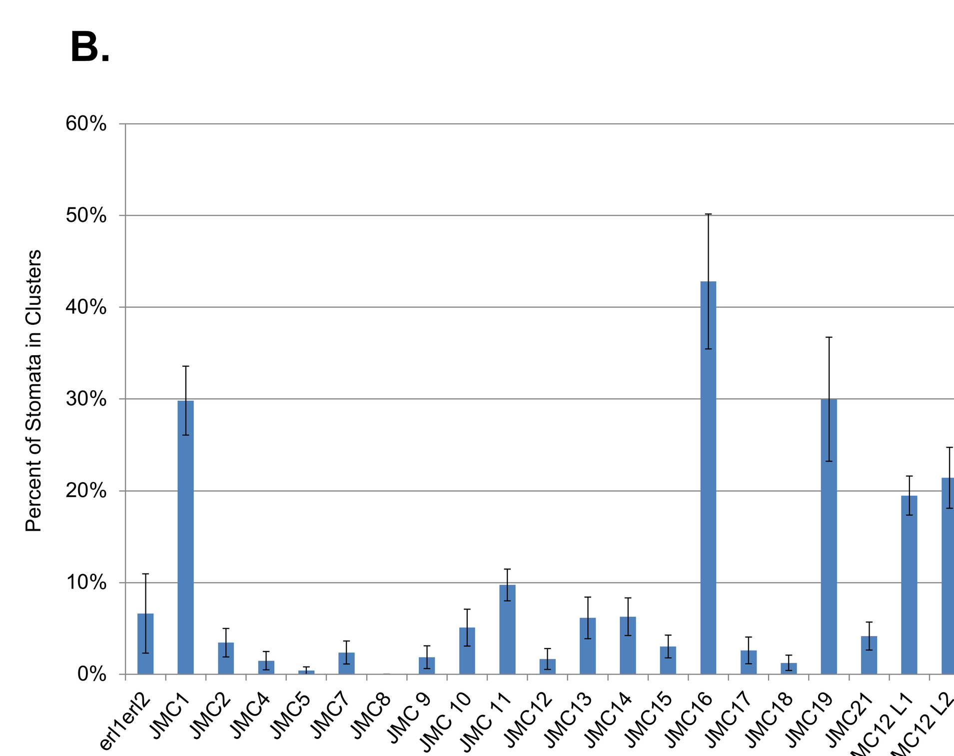


An enhancer genetic screen was used to identify mutant phenotypes that might otherwise be weak and unobservable in a regular genetic screen. The identified mutants were then crossed with other ecotypes and background mutations in order to better characterize the mutant genotype and phenotype. Lastly, the mutants are crossed with an ecotype (Ler) in which specific genetic markers are known. Based upon recombination frequency the position of the mutated gene(s) can be pinpointed.

Identifying Mutants

Figure 1. Search for Mutants in M_2





C.

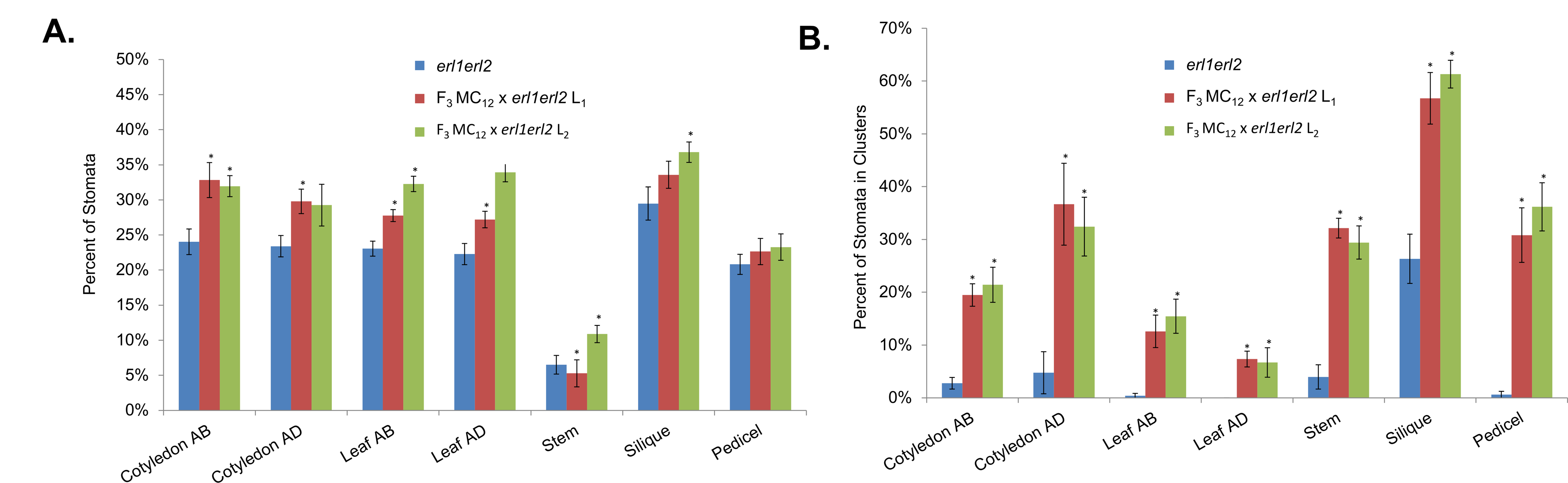
Adaxial (AD)

Abaxial (AB)

(A) The initial screening for mutants was performed by microscopically observing the abaxial side of one cotyledon per seedling. The mutants were selected based upon their strong phenotype. (B) From all of the selected mutants, JMC_{19} and MC_{12} were chosen to pursue further because of their strong mutant phenotype in comparison to the control, *erl1erl2*. JMC_1 and JMC_{16} were lost due to decreased fertility. (C) Abaxial is the bottom side of the cotyledon and adaxial is the top.

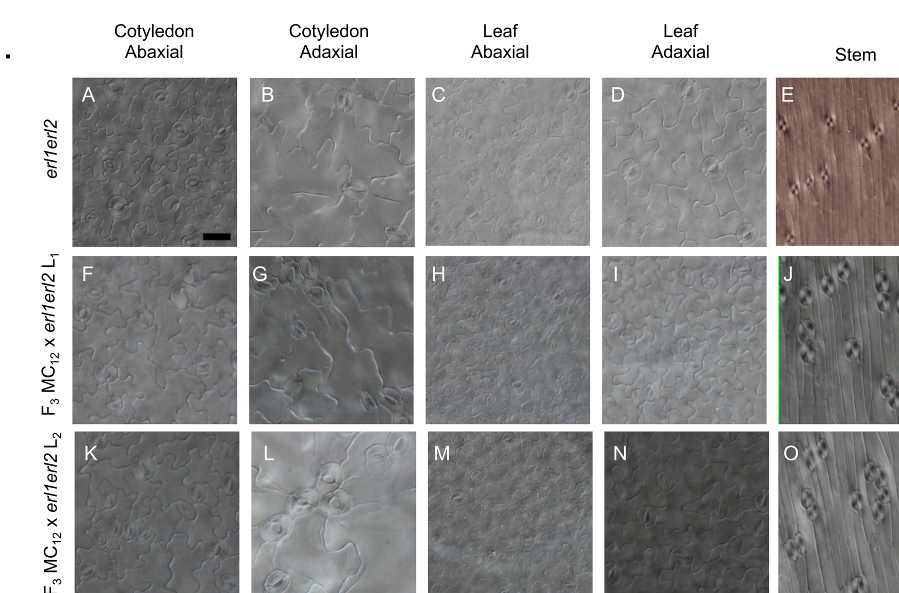
Characterization of The Mutant Phenotype

Figure 2. MC_{12} Phenotype Shows An Increase in Stomata Density and Clustering



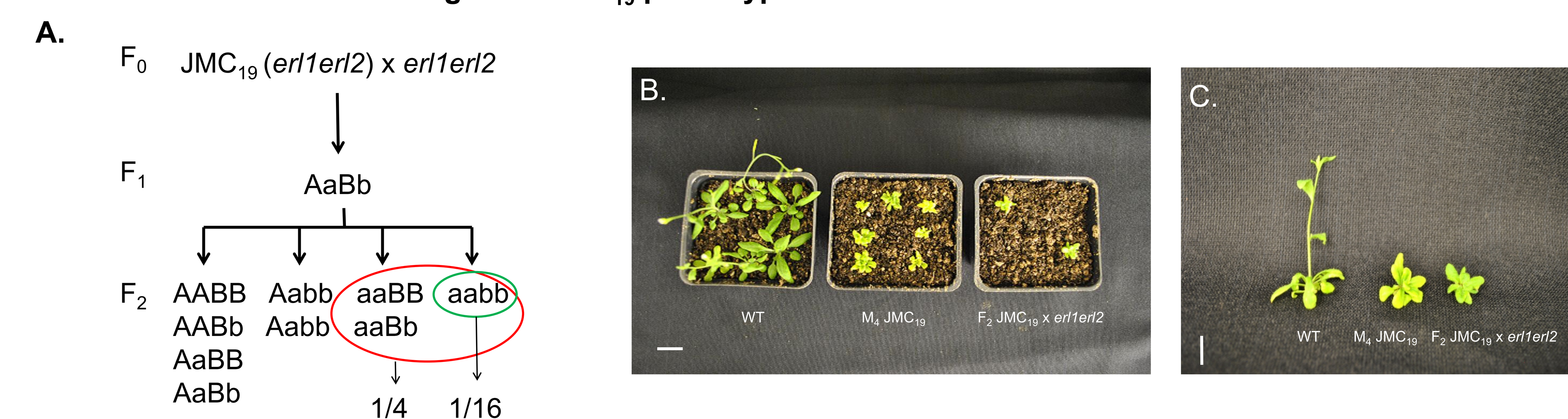
MC_{12} was characterized by calculating stomata index (the number of stomata out of the total number of stomata and pavement cells) and percent of stomata in clusters. A, MC_{12} has a higher percentage of stomata in clusters compared to *erl1erl2*. B, Most organs of MC_{12} show a statistically significant difference in stomata index from *erl1erl2*. Differential interference contrast (D.I.C) microscopy was used to collect the data. In A and B, n = 6 to 12 and error bars represent standard error. Values significantly different from the control (*erl1erl2*) are denoted by asterisks (P < 0.05).

Figure 3. MC_{12} shows a significant difference in stomata index and percent of stomata in clusters



The MC_{12} mutant phenotype can be observed in cotyledons (AB and AD), first and second leaves (AB and AD), and stems. There is a significant difference in the stomata index and percent of stomata in clusters in MC_{12} when compared with the control, *erl1erl2*. A to O, pictures were taken using differential interference contrast (D.I.C.) microscopy. Bar = 5 μm

Figure 4. JMC_{19} phenotype is due to two recessive mutations



(A) Preliminary data suggest that JMC_{19} phenotype occurs because of two recessive mutations. When one mutated gene is present, a weak stomata clustering phenotype is observed (aaBB or aaBb). When other mutated gene is present (bb) plants are short. Two mutated genes (aabb) yield a strong stomata clustering and short stature phenotype in 6.25% of F_2. (B and C) This shows that A and B genes synergistically regulate stomata development. Bar = 2 cm in B and C.

Future Directions

- -Determine if MC_{12} is a dominant or recessive mutation(s) and whether it function synergistically with erl1 and erl2
- -Verify that JMC_{19} phenotype is caused by two mutations and analyze whether those two mutations function synergistically with known mutations affecting stomata development
- -Conduct positional cloning for JMC_{19} and MC_{12}

References

[1]**Bergman DC, Sack FD** (2012) Stomatal development: a plant's perspective on cell polarity, cell fate transitions and intercellular communication. Development **139**: 3685

[2]**Shpak ED, McAbee JM, Pillitteri LJ, Torri KU** (2005) Stomatal Patterning and Differentiation by Synergistic Interactions of Receptor Kinases. Science **309**: 290-293