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 M2 seeds were grown and their seeds collected. M3 seeds were grown and plants were selected for the mutant phenotype (stomata clusters).

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 stomatal.

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 mutant has a phenotype of short height, short siliques, and compact inflorescence. When a mutant has either erl1 or erl2 mutations only, the expressed phenotype is drastically weaker. Double erl1 erl2 and erl1 mutants have even stronger phenotypes and shorter in stature. When all three genes are mutated (erl1 erl2), the plant is extremely short and compact. Over-proliferation of stomata and development of stomatal clusters is evident in the triple mutant.

ERL1 and ERL2 Act Synergistically With ERECTA

Figure 1. Search for Mutants in M3

Mutants x erl1erl2
-Characterization of Mutant Phenotype
-Dominant or Recessive
-1 or 2 Mutations
-Synergistic Interaction

Mutants x Col
-Dependence of Mutation(s) On erl1 and/or erl2

Mutants x Ler
-Positional Cloning

Identifying Mutants

Figure 1. Search for Mutants in M3

Approximately 10,000 Arabidopsis T. seeds of erl1erl2 were mutagenized with ethyl methanesulfonate (EMS). The mutagenized M2 seeds were grown and their seeds collected. M3 seeds were grown and plants were selected for the mutant phenotype (stomata clusters).

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 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.

ERL1 and ERL2 Act Synergistically With ERECTA

Figure 2. MC1 Phenotype Shows An Increase in Stomata Density and Clustering

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 controls this developmental process.

Figure 3. MC1 shows a significant difference in stomata index and percent of stomata in clusters

Future Directions

- Determine if MC1 is a dominant or recessive mutation(s) and whether it function synergistically with erl1 and erl2
- Verify that MC1 phenotype is caused by two mutations and analyze whether those two mutations function synergistically with known mutations affecting stomata development
- Conduct positional cloning for MC1

References


Figure 4. JMC19 phenotype is due to two recessive mutations

(A) Preliminary data suggest that JMC19 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 62.5% of F3, (B) and (C) This shows that A and B genes synergistically regulate stomata development. Bar = 2 cm in B and C.

Results

Characterization of The Mutant Phenotype

The MC1 mutant phenotype 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, MC1 has a higher percentage of stomata in clusters compared to erl1erl2. B, Most organs of MC1 show a statistically significant difference in stomata index from erl1erl2. Differential interference contrast (DIC) 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).

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