EPFL Genes And Their Role in Flower Development in *Arabidopsis thaliana*

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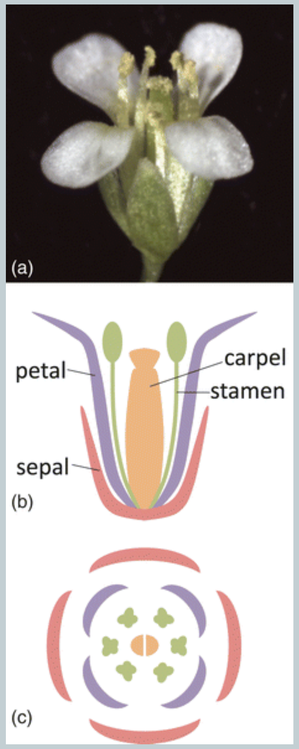
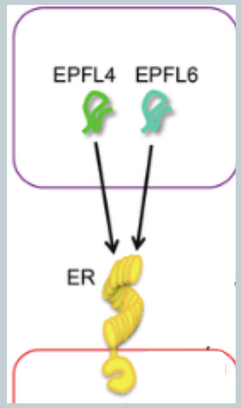
BACKGROUND

Many aspects of plant development are facilitated through intercellular communications. Membrane-localized leucine rich repeat receptor-like kinases (LRR-RLKs) sense a variety of molecules and initiate signaling processes necessary for responses to environmental stimuli and development. Since the pathways of these receptors involve many interconnected components and are often affected by genetic redundancy, many signaling processes remain a mystery. In *Arabidopsis thaliana*, the ERECTA family (ERf) LRR-RLKs have been shown to regulate plant morphology. They were first linked to aboveground organ elongation (Torii et al., 1996), and were later discovered to regulate flower differentiation and the development of reproductive tissues (Shpak, 2013). Epidermal Patterning Factor-Like (EPFL) genes encode for small secretory proteins that are ligands for ERECTA Family (ERf) receptors. It is suspected that EPFL's act as a signal to coordinate proper lateral organ number, patterning, and spacing. ERf mutants have significant defects in flower development, including difficulty forming anther lobes and pistils, yet little is known about how individual EPFL ligands contribute to ERf signaling. EPFL4 and EPFL6 have been shown to stimulate aboveground organ elongation (Uchida et al., 2012), and the mutation of EPFL1 and EPFL2 in the *epfl 4,6* mutant background displays a significant decrease in plant height and difficulty properly forming lateral organs (Kosentka et al., 2019). Various *epfl* mutant combinations were analyzed with the goal of utilizing flower development as a model system to understand the distinct roles of individual EPFL ligands and, ultimately, ERf signaling pathways.



Irish, 2010

Figure One.
The *Arabidopsis thaliana* Flower. Flowers are composed of four sepals, four petals, six stamens, and a pistil.
(A) Mature flower at antithesis.
(B) Diagram of lateral section through mature flower depicting floral organs.
(C) Floral diagram showing placement of organs.



Tameshige, 2016

Figure Two.
An illustration of an ERECTA family LRR-RLK and two EPFL ligands.

Figure Three.
Tissues of the Stamen.
The stamen, which consists of a four-lobed anther and a filament, is the male reproductive organ. This photo of a Wild Type stamen at floral stage 11 was taken using DIC microscopy at 10X magnification. The image is falsely colored to illustrate the different tissues: green=anther and yellow=filament.

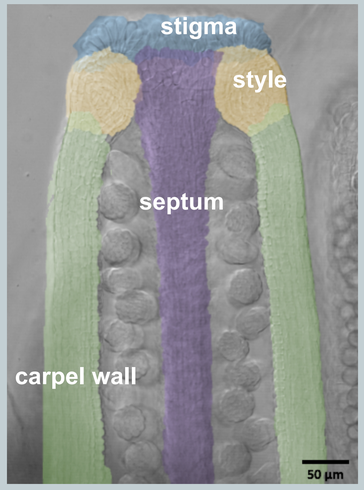


Figure Four. Tissues of the Pistil.
The pistil is the female reproductive organ. The stigma (blue) captures pollen, which is then directed down the transmitting tract, which is embedded in the septum (purple), to fertilize ovules. This photo was taken of cleared tissue of a WT pistil (floral stage 11) using DIC microscopy at 20X magnification. It was then falsely colored to illustrate the different tissue types: Green=carpel walls, purple=septum, yellow=style, and blue=stigma.

RESULTS

Floral Organ Lengths May Be Negatively Affected by the Loss of EPFL Genes

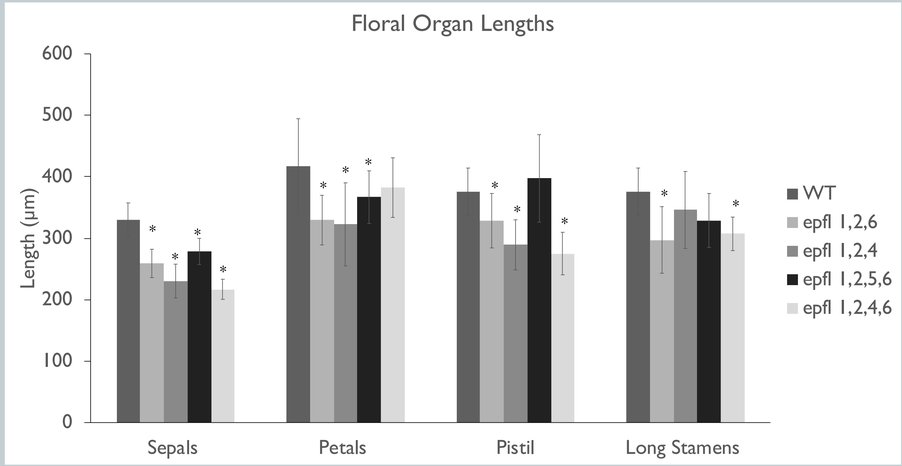


Figure Five. Floral Organ Lengths in epfl Mutants.
A Nikon Eclipse 80i camera and DIC (Differential Interference Contrast) microscope were used to take length measurements of floral organs. This was accomplished using NIS-Elements BR imaging software at a magnification of 4X. Fifteen flowers of Wild Type (Columbia ecotype), two epfl triple mutants, and two epfl quadruple mutants were analyzed. One measurement was taken per organ type in each flower, and the values were averaged. The data suggests that floral organ lengths are negatively affected by the loss of EPFL genes. T-Test compared to WT at $p<0.05$. This experiment should be repeated to ensure that similar stages of flower development were selected.

EPFL1, 2, 4, and 6 Synergistically Regulate Number of Anther Lobes

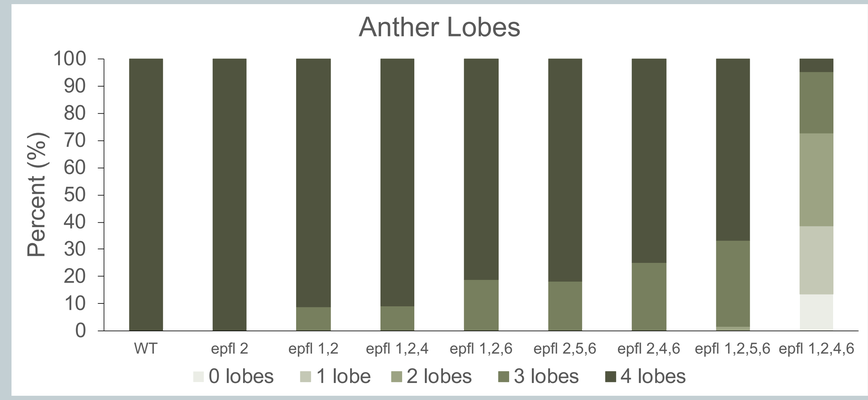


Figure Six. Number of Anther Lobes in epfl Mutants.
Tissue clearing methods and DIC microscopy were utilized to count the number of anther lobes in WT and epfl mutant stamens. All six stamens (two short and four long) were analyzed in eight randomly selected flowers (floral stages 11-13) for each type. Four anther lobes were detected in 100% of the inspected stamens for WT and *epfl 2*, but the 3-lobe phenotype appears in *epfl 1,2* and the triple mutants. On rare occasion (1.6%) only two anther lobes are formed in *epfl 1,2,5,6*. *epfl 1,2,4,6* displayed the most difficulty forming lobes and had an even distribution in the number of anther lobes formed.

EPFL1 and 2 Synergistically Regulate Elongation of the Style

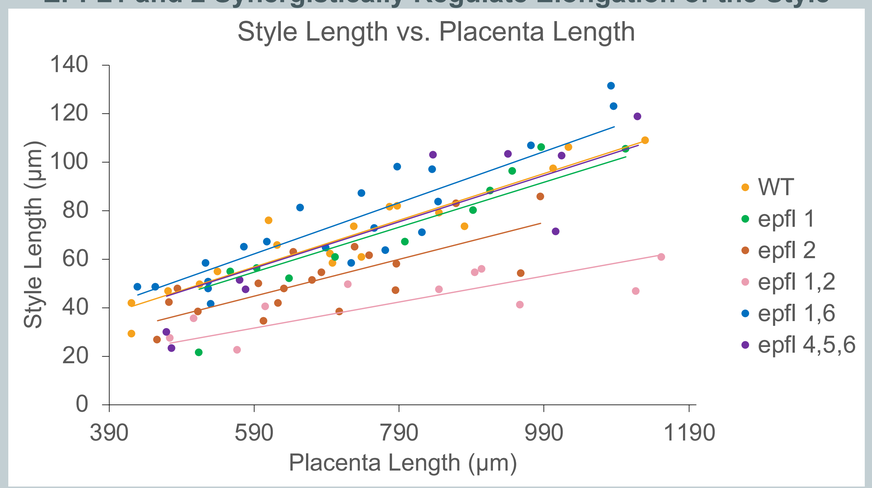


Figure Seven. Ratios of Style Length to Placenta Length in epfl Mutants.
DIC microscopy and tissue clearing techniques were used to take length measurements of the style and carpel valve (placenta) lengths in the epfl mutants (12-18 flowers each) at varying developmental stages (floral stage 8-15). The results captured the change in style length in relation to the placenta length throughout development. The regression line of *epfl 2* shows a decrease in style elongation throughout development, and even more so in *epfl 1,2*. Slope intercepts of the regression lines were set to 0.

CONCLUSIONS

The data suggests that when a plant is missing EPFL1 and EPFL2 errors occasionally occur during development of reproductive tissues (Fig. 6 and 7), and that triple mutants missing either EPFL1 or EPFL2 in addition to missing EPFL 6 are more likely to encounter developmental problems, whether it be proper anther lobe formation or the elongation of floral organs (Fig. 5 and 6). Ultimately, the data supports the claim that, while EPFL 1, 2, 4, and 6 in congruence are crucial for the correct developmental processes, EPFL1 and 2 have unique roles in organ development, especially in the style.

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