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ERECTA family genes regulate development of cotyledons during embryogenesis

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ABSTRACT

Receptor-like kinases are important regulators of plant growth. Often a single receptor is involved in regulation of multiple developmental processes in a variety of tissues. ERECTA family (ERf) receptors have previously been linked with stomata development, above-ground organ elongation, shoot apical meristem function, flower differentiation and biotic/abiotic stresses. Here we explore the role of these genes during embryogenesis. *ERfs* are expressed in the developing embryo, where their expression is progressively limited to the upper half of the embryo. During embryogenesis *ERfs* redundantly stimulate the growth of cotyledons by promoting cell proliferation and inhibiting premature stomata differentiation.

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1. Introduction

During development a multicellular organism must coordinate the proliferation pattern and specialization behavior of its cells. Cell cooperation in such an organism is dependent upon communications between cells mediated by molecular messages. Receptor-like kinases are primarily plasma membrane localized proteins that can sense extracellular molecules. Many of these receptors are involved in sensing signals originating from other cells.

ERf receptor-like kinases appeared early in land plant evolution and already existed in the time of bryophytes [1]. All analyzed angiosperms have at least two genes belonging to this family, but in many plant species further gene duplications occurred. Arabidopsis has three such genes: *ERECTA* (*ER*), *ERECTA LIKE1* (*ERL1*) and *ERECTA LIKE2* (*ERL2*) [2]. These ERf receptors sense secreted cysteine-rich peptides from the EPF/EPFL family, enabling cell–cell communications in various plant tissues [3].

The ERf family is involved in regulation of multiple aspects of plant development [4]. ERf receptors regulate the growth of above-ground plant biomass where they promote organ elongation, shoot apical meristem function, and cell specification in the epidermis. The *er* mutants have a compact inflorescence due to reduced elongation of internodes and pedicels, round flowers, and short siliques and petioles [5,6]. A temporal study of pedicel development demonstrated that during early stages of organ growth ER promotes elongation of epidermal and cortex cells along the proximodistal axis which accelerates the cell cycle [7]. The elongation of above-ground organs is further reduced when ERL1 and ERL2 are mutated in the *er* background [2]. But the most dramatic phenotype is observed in the *er erl1 erl2* mutant. In addition to severely reduced organ elongation, the *er erl1 erl2* mutant has novel phenotypes that cannot be observed in single or double mutants. The *er erl1 erl2* plants are sterile; in their flowers the stem cell population is often misplaced, ovules and anthers do not differentiate properly, and sepals often have stigmatic papillae on top [2,8]. This homeotic conversion of sepals might be a consequence of ectopic expression of the transcription factor AGAMOUS [8]. The *er erl1 erl2* seedlings also have bigger shoot apical meristems (SAM) and form leaves at a slower rate and with abnormal phyllotaxy [9,10]. The changes in SAM structure and function correlate with increased sensitivity to cytokinins and with changes in auxin distribution and transport [9,10]. Finally, an analysis of *er erl1 erl2* epidermis demonstrated

Abbreviations: SAM, shoot apical meristem; ERf, ERECTA family; ER, ERECTA; ERL1, ERECTA LIKE1; ERL2, ERECTA LIKE2; MMC, meristemoid mother cells; GMC, guard mother cell; TMM, TOO MANY MOUTHS

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that ERfs inhibit stomata formation and regulate their spacing [11]. While multiple functions of ERfs have been described, the involvement of this gene family in regulation of embryogenesis has not been investigated prior to this study.

Plant embryogenesis can be divided into two phases: morphogenesis and maturation. During the first phase three tissue layers (the protoderm, ground tissue, and procambium) are formed and the basic body plan is created. During the second phase the embryo grows and prepares to enter metabolic quiescence. In Arabidopsis a series of stages named after the shape of the embryo is recognized: preglobular, globular, heart, torpedo, and walking stick [12]. The morphogenesis phase of embryogenesis continues until the heart stage when patterning of embryo is completed and the shoot apical meristem, the cotyledons, the hypocotyl, root, and the root apical meristem are established. The maturation phase begins during the heart stage when proplastids in the epidermis of hypocotyls differentiate into chloroplasts [13]. Analysis of *er erl1 erl2* embryo development demonstrated that beginning from the heart stage ERfs promote cotyledon growth and inhibit differentiation of epidermis.

2. Results

2.1. Expression of ERf genes during embryo development

Analysis of microarray data [14] suggests that *ERfs* are expressed in the embryo proper beginning from the globular stage and tapering off by the end of embryo maturation. To verify these data we analyzed the expression of *ER*, *ERL1*, and *ERL2* promoter-GUS fusions [2]. As anticipated, activity of all three promoters was detected in the embryo proper from the globular stage to the maturation stage (Fig. 1). In the globular embryo the genes were expressed everywhere except in cells originated from the hypophysis. Beginning from the heart stage, the activity of all three promoters was consistently limited to the upper half of the embryo (Fig. 1B, G, and L). This confinement of *ERf* gene expression to the apical end became especially noticeable during the early torpedo stage (Fig. 1C, H, and M). In the early walking stick stage the expression of *ERf* genes is restricted to the shoot apical meristem (SAM) and cotyledons (Fig. 1D, I, and N), and later in that stage it subsides, with the longest expression in the SAM (Fig. 1E, J, and O).

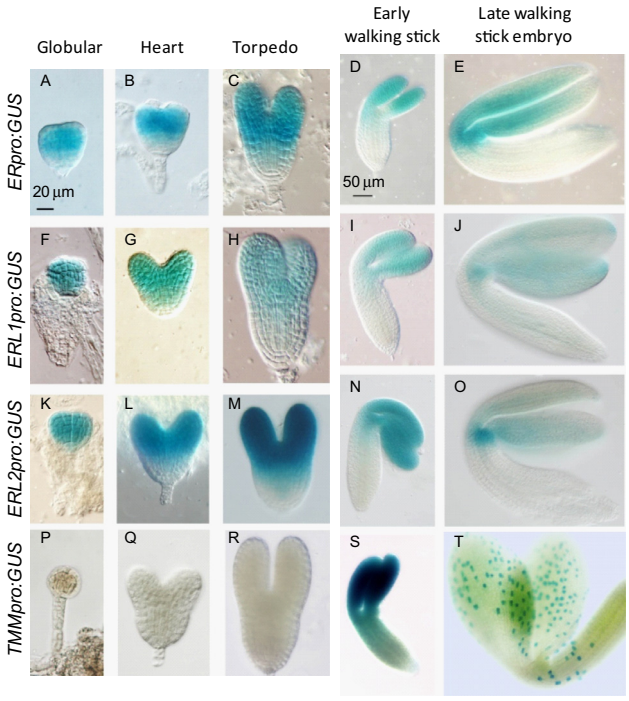

Fig. 1. Transcriptional reporters of *ERfs* are expressed during embryogenesis in the upper portion of the embryo. The promoters of *ER*, *ERL1*, *ERL2*, and *TMM* were fused to *GUS* and their activity was monitored in developing embryos. Bar = 20 μm for (A) and bar = 50 μm for (D). Images for (A)–(C), (F)–(H), (K)–(M), (P)–(R), and images (D), (E), (I), (J), (N), (O), (S), and (T) are at the same magnification. Stages of embryo development are indicated on top.

TOO MANY MOUTHS (TMM) is a receptor-like protein that is able to form heterodimers with ERfs [15]. It is known to be expressed in shoot epidermal cells [16]. Our analysis of TMM expression demonstrated that during embryogenesis TMM is expressed much later compared to ERfs, at the end of the torpedo stage or in the early walking stick embryo, mostly in developing cotyledons (Fig. 1S). In a short time its expression becomes limited to stomatal lineage cells which has not been previously observed for ERfs (Fig. 1T). Thus, all four genes analyzed are expressed during embryogenesis, preferentially in the upper part of the embryo. And while there is an overlap in their expression, the expression pattern of TMM and ERfs are quite distinct.

2.2. ERfs stimulate cotyledon growth during embryogenesis

High expression of *ERf* genes in the developing embryo prompted us to investigate the role these genes play during embryogenesis. Since *ER*, *ERL1*, and *ERL2* are expressed in a highly overlapping pattern at this stage of plant development, we anticipated some redundancy in their function. Consequently, we did not detect a conspicuous phenotype in single or double mutants. As *er erl1 erl2* mutants are infertile, we analyzed embryogenesis in *er erl2 erl1*/+ plants and compared them to the wild type. We observed that 26.6% of embryos (N_{total} = 94) produced by *er erl2 erl1*/+ plants developed differently beginning from the early torpedo stage, and these embryos were considered to be *er erl1 erl2* triple mutants. In these embryos, cotyledons grew more slowly than the axis and never reached the expected size (Fig. 2C, F, I, and L). In the WT the length of cotyledons from the torpedo stage to embryo maturity is correlated with the length of the axis and amounts to 60–75% of it (Fig. 2M). In the triple mutant the length of cotyledons was only 30–40% of the axis length. We did not observe this phenotype in any WT embryos, and the remaining 73.4% of embryos developing from *er erl2 erl1*/+ plants were indistinguishable from the WT (Fig. 2N). At the end of embryo growth, in the late walking stick stage, the cotyledons of mutant embryos were considerably shorter (234 ± 8 μm; mean ± standard deviation here and below) than either WT (353 ± 18 μm) or their siblings (351 ± 23 μm), while their axis was longer (618 ± 25 μm in mutants versus 535 ± 27 μm in siblings and 471 ± 25 μm in WT). The phenotype observed in *er erl1 erl2* was not present in *tmm-1* embryos. The *tmm-1* embryos had a regular shape where the fractional length of cotyledons to axis was 72 ± 6% (n = 10, measured in early walking stick stage). Previously it has been reported that ERfs regulate SAM size [9,10]. Analysis of *er erl1 erl2* embryo development suggest that the SAM size is already increased during early stages of embryogenesis (compare Fig. 2A and C). However, decreased growth of cotyledons in *er erl1 erl2* is not likely to be a direct consequence of changes in SAM size. In *clv3* mutants the embryo SAM size is increased [17] but our analysis of *clv3-9* embryogenesis did not detect any changes in cotyledon elongation and the ratio of cotyledon to axis length was 71.7 ± 7.5% (n = 10, measured in the late walking stick stage).

2.3. ERfs promote cell proliferation in cotyledons

The decreased size of *er erl1 erl2* cotyledons was easily noticeable immediately after seed germination (Fig. 3A and B). To

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