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Arabidopsis mitochondrial protein slow embryo development1 is essential for embryo development

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ABSTRACT

The plant seeds formation are crucial parts in reproductive process in seed plants as well as food source for humans. Proper embryo development ensure viable seed formation. Here, we showed an *Arabidopsis* T-DNA insertion mutant *slow embryo development1* (*sed1*) which exhibited retarded embryogenesis, led to aborted seeds. Embryo without SED1 developed slower compared to normal one and could be recognized at early globular stage by its white appearance. In later development stage, storage accumulated poorly with less protein and lipid body production. *In vitro* culture did not rescue albino embryo. *SED1* encoded a protein targeted to mitochondria. Transmission electron microscopic analysis revealed that mitochondria developed abnormally, and more strikingly plastid failed to construct grana in time in *sed1/sed1* embryo. These data indicated that SED1 is indispensable for embryogenesis in *Arabidopsis*, and the mitochondria may be involved in the regulation of many aspects of seed development.

1. Introduction

Plant embryogenesis is a complex developmental process that takes place just after fertilization. During embryogenesis, the zygote undergoes a series of morphological and cellular changes to form an embryo, which marks the beginning of the diploid phase of the flowering plants' life cycle. Embryogenesis is considered generally with establishing the basic shoot-root body pattern of the plant and accumulating food for seed germinating [1–4].

A detailed understanding of the events that govern plant embryo formation is yet to be realized. One approach to identify genes required for various embryonic processes is the isolation and characterization of mutants that have defects in embryo development. Several embryo-defective mutants have been isolated in *Arabidopsis* and other species. On the one hand, a series of genes are involved in establishing the pattern of the embryo cell patterning. TOZ affects longitudinal cell divisions and its loss-of-function mutants result in arrest embryo defects [5]. WDR55-DDB1 complex is involved in the establishment of bilateral symmetry in the *Arabidopsis* embryo [6]. The *Arabidopsis* Miro GTPase EMB2473/MIRO1 is required for early stage embryogenesis between the zygote and the four-terminal-cell stage and influences mitochondrial morphology in pollen [7]. CLE8 regulates embryo and suspensor proliferation by ensure the right plane of cell division in uppermost suspensor cells [8]. POD1 plays a specific role in the micropylar response and is also essential for cell patterning during early embryogenesis in *Arabidopsis* [9]. *Arabidopsis* mitochondrial protein Oxa1a and Oxa2 function in embryo formation and are indispensable for plant development [10]. On the other hand, lots of genes are used for massive reserves synthesis and accumulation before desiccating to form a seed [11]. Mutation of GE results in a large embryo in the seed, which is caused by excessive expansion of scutellum cells in rice [12]. TAN interacts with other proteins to control many aspects of embryo development and *tan* mutant embryos have defects in protein and lipid body accumulation [13]. Mutations in the ABA insensitive gene *abi3*, *abi4* and *abi5* also cause defects in storage protein accumulation [14–16]. All above genes have been shown to be required for proper embryo development since their mutants exhibit aberrant cell divisions, the reduction of nutrition accumulation and are generally embryo-lethal. In addition, other reasons not easily classifiable for defective embryo development also exist.

Abbreviations: SED, slow embryo development.

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ETHE1, which is localized in the mitochondrion and exhibits sulfur dioxygenase activity, is essential for the formation of enough endosperm nuclei, thus for normal embryo development [17]. AESP plays an essential role in embryo development by removal of cohesion from meiotic chromosomes [18]. A large dataset of *embryo defective* (*emb*) genes required for normal embryo development have been identified and appear to function in different basic cellular networks.

In this paper, we demonstrate a novel *Arabidopsis* embryo-defective mutant called *slow embryo development1* (*sed1*), which has less protein and lipid body in the embryo cells. The *sed1* embryo exhibits arrested development by the fourth day post-pollination and a white appearance throughout the whole developmental process, thus leading to no seed germination. Taken together, these results define that SED1 is essential for nutrition accumulation during embryogenesis in *Arabidopsis*.

2. Materials and methods

2.1. Plant materials and growth conditions

The heterozygous T-DNA insertion mutant line (SALK_043472C, TAIR: At1g55040) was obtained from *Arabidopsis* Biological Resource Center (Columbus, OH) and designated *sed1*. Wild type Columbia (Col-0) was used as the control in development studies. The genotypes of these lines were confirmed by PCR amplification using gene-specific primers (sed1-LP, 5′-AGATGTGCC-TAGCTTCTGCTG-3′ and sed1-RP, 5′-AGCTTAGGGCTTTGAAATTCG-3′) and T-DNA–specific primers (LBa1, 5′-TGGTTCACGTAGTGGGC-CATCG-3′). The template DNA was prepared by the method of Klimyuk [19]. PCR was performed at 94 °C for 3 min followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, and another stage at 72 °C for 10 min. Seeds and ovules (dissected under the microscope in sterile conditions for *in vitro* culture) were surface-sterilized and plated onto 0.8% (w/v) agar medium containing half-strength MS salts (pH 5.8; Sigma–Aldrich) and 3% sucrose, which were supplemented with 50 mg/L kanamycin as required. The seeds were vernalized at 4 °C for 48 h and germinated, and the 10-d-old seedlings were transferred onto commercial potting soil in an air-conditioned room at 22 °C under a 16-h-light/8-h-dark regime.

2.2. Molecular analysis and plasmid strains

To test for complementation of the mutant, we generated the construct pGreen-proSED1-SED1-GFP, which contained 519 bp upstream from the ATG and a 2550 bp fragment encompassing the coding sequence, and the green fluorescent protein, and it was subcloned into the pGreen vector using *Kpn*I-*Sal*I, *Sal*I-*Xba*I restriction sites, respectively. The promoter region was amplified from Col-0 genomic DNA with primers (pro-sed1-F, 5′-GCGGGTACCGATTAGAATCCAGGAAAAAAAATT-3′ and pro-sed1-R, 5′-TATGTCGACTTTTAGGTTGCAGAAGCAGC-3′). The *SED1* cDNA sequence was amplified using *Arabidopsis thaliana* flower cDNA as template with primer pairs (sed1-F, 5′-TTGGTCGA-CATGGCTGCTTCAATCTCTC-3′ and sed1-R, 5′-GCGTCTA-GACTATCTCTCGATAACTC-3′). *Agrobacterium tumefaciens* strain GV3101 was used to stably transform *Arabidopsis* plants by the dip method into *sed1* mutants and Col-0 [20], resulting homozygous seeds termed *SED1-RES* and *SED1-OE*. Similarly, the proSED1-GUS was made into pBI121 by *Hind*III and *Xba*I and transformed into *Arabidopsis* plants.

2.3. Subcellular localization assay

Plant mitochondria were visualized in plants transformed with a vector containing Lat52 promoter, Dips sequence and the RFP sequence. To identify the subcellular localization of SED1, *SED1-OE* crossed to mitochondrial marker line *Lat52-Dips-RFP*, and three independent T1 plants were used for the microscopic analysis.

2.4. Electron microscopy

Embryos from heterozygous *sed1* plants were dissected from ovules by forceps and then performed as previously described [21].

2.5. Microscopy

Embryos and endosperms were viewed by directly mounting seeds in clearing solution (8:2:1 chloral hydrate:deionized water:glycerol). After 2 h of incubation at room temperature, embryo and endosperm development could be viewed under an epi-fluorescence microscope (DMI 6000B; Leica) equipped with a CCD camera (DFC480; Leica).

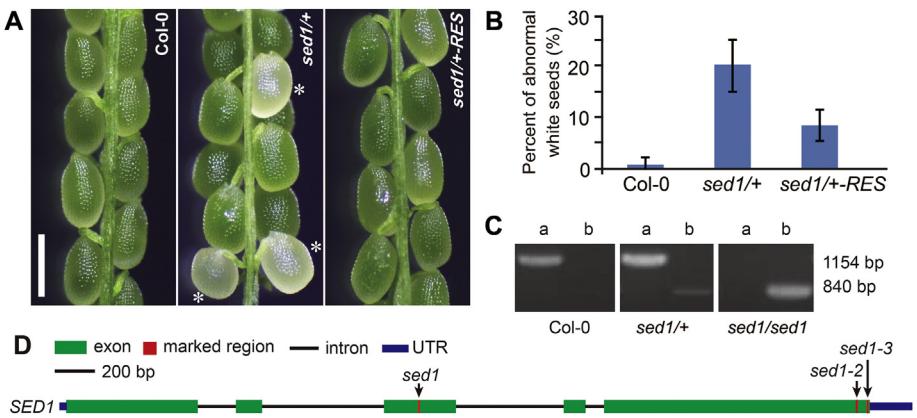

Fig. 1. T-DNA insertional mutant of SED1. (A) Siliques from Col-0, *sed1*/+ and *sed1*/+-*RES* plants. The heterozygous mutant *sed1*/+ have white seeds with reduced rates of development (middle panel, indicated by asterisks). Silique from *sed1*/+-*RES* (*sed1*/+ plants complemented by the *SED1* cDNA driven by the native promoter) rescued the phenotypes. Bars = 500 μm. (B) The percentage of abnormal white seeds in Col-0, *sed1*/+, and the *sed1*/+-*RES* plants. (C) Confirmation of the T-DNA insertion lines by PCR. Primers sed1-LP and sed1-RP were used in Lane a, and primers Lba1 and sed1-RP were used in Lane b. See Methods for details. (D) SED1 gene structure and the three T-DNA insertional mutant. The positions of exons are shown as green boxes. The positions of T-DNA insertions are shown as red lines.

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