Argon-ion-induced mutant of *Arabidopsis thaliana* exhibiting accelerated leaf chlorosis†

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Chloroplast development in leaf tissues is crucial for plant growth and productivity. Plant leaves are composed of the epidermis, which forms the outermost layer, and the mesophyll and vasculature inside. In the model plant *Arabidopsis thaliana*, chloroplasts are distributed in leaf epidermal pavement and guard cells, as well as in mesophyll cells. Despite vast research on the structure and function of leaf chloroplasts, there is limited knowledge about their regulations in the plant life cycle.

To explore possible novel gene functions for leaf chloroplast development, we attempted a forward genetic approach. We created mutants of *A. thaliana* by exposing dry seeds of ecotype Col-0 to accelerated argon ions (290 keV/μm, 50 Gy) at RIBF.[1)] Following two rounds of cultivation and selfing, as well as a macroscopic screening of the M_3 generation of plants, we isolated a new mutant, designated as Ar50-33-pg1.

During seedling development, Ar50-33-pg1 produced slightly pale cotyledons and leaves under standard plant growth conditions (Fig. 1A, B). As the mutant leaves expanded and matured, their chlorosis became prominent (Fig. 1C). This observation of accelerated leaf chlorosis was supported by the chlorophyll measurement of detached leaves through spectrophotometry (data not shown). Furthermore, Ar50-33-pg1 exhibited abnormalities at the reproductive stage, including phenotypes of late flowering, flower longevity, and low seed production (data not shown).

Microscopic examination of expanding leaves of wild-type and Ar50-33-pg1 plants under normal observation conditions revealed that a certain population of mutant epidermal chloroplasts lacked chlorophyll autofluorescence signal (Fig. 2). Meanwhile, mutant mesophyll chloroplasts retained chlorophyll autofluorescence until later stages of leaf senescence. Therefore, the chlorosis leaf phenotype of Ar50-33-pg1 might involve tissue-dependent chloroplast abnormalities between the epidermis and mesophyll.

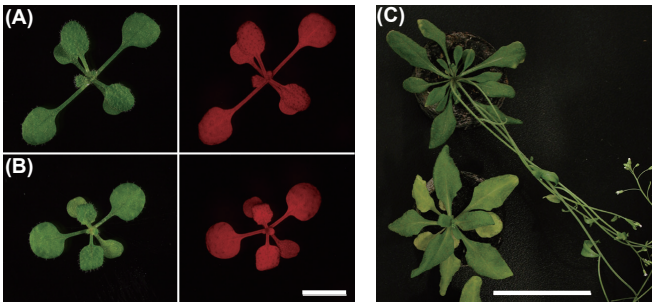

Fig. 1. Growth and morphology of seedlings of *Arabidopsis*. (A) 2-week-old wild-type seedling. (B) 2-week-old Ar50-33-pg1 seedling. In (A) and (B), images of chlorophyll autofluorescence were taken at the same excitation condition by stereofluorescence microscopy. Scale bar = 5 mm. (C) 4-week-old wild-type and Ar50-33-pg1 seedlings. Scale bar = 5 cm.

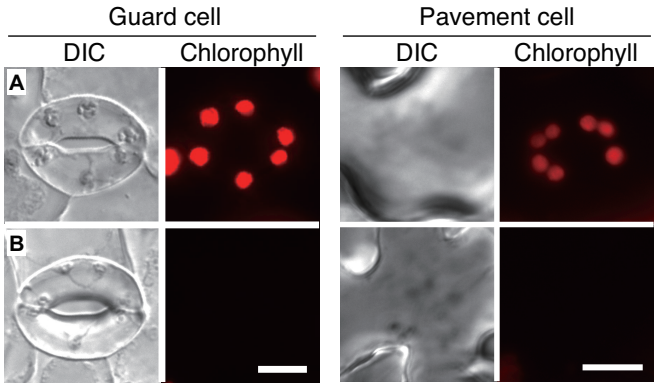

Fig. 2. Fluorescence microscopy of leaf epidermal guard cells and pavement cells of *Arabidopsis*. (A) Wild type. (B) Ar50-33-pg1. Differential interference contrast (DIC) and chlorophyll autofluorescence (colored in red) images of cells from epidermal peels of growing rosette leaves. Scale bar = 10 μm.

Backcrossing and segregation analyses indicated that the chlorotic phenotype of Ar50-33-pg1 is associated with impaired epidermal chloroplasts and is caused by a single nuclear recessive allele. Through whole-genome resequencing, Ar50-33-pg1 was revealed to contain a ~0.9 Mb deletion from position 12621534 to 13561533 in chromosome V, which spans approximately 40 putative or well-characterized protein-coding genes. One of them encoded a known chloroplast membrane-localized, ATP-independent metalloprotease, Ethylene-dependent Gravitropism-deficient and Yellow-green 1 (EGY1).[2)]

Ar50-33-pg1 was then crossed with an *egy1* mutant[3)] in both directions. All F_1 seedlings were similar to both parents in that they commonly exhibited accelerated leaf chlorosis with epidermal chloroplast defects. F_1 plant phenotypes at the reproductive stage were all wild type-like. Therefore, *EGY1* was deemed to be the main causal gene for the impaired chloroplast phenotypes in Ar50-33-pg1.

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