S41-008

Characterization of a high chlorophyll fluorescence mutant of *Arabidopsis thaliana*

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Keywords: *Arabidopsis thaliana*, Chlorophyll fluorescence, hcf mutant, T-DNA tagging

Introduction

Quite a large proportion of mutations in plastid gene expression resulted in high chlorophyll fluorescence (hcf) phenotype, because photosynthetic electron flow in these mutants were impaired. Therefore, screenings for high chlorophyll fluorescence have been utilized to isolate nuclear mutants which showed abnormal gene expression in chloroplasts (for review, see Barkan 1998).

Interestingly, a number of hcf mutants so far reported showed abnormality in processing or stability of RNA from specific plastid genes, while transcription mutants were very few, suggestive of the importance of the nuclear regulation at post-transcription step in plastid gene expression (for review see, Monde et al. 2000). However, identity of gene or protein directly involved has not been clarified yet in most of these mutants. In order to isolate nuclear genes involved in plastid gene expression, screening of Arabidopsis seedlings for high chlorophyll fluorescence have been newly conducted (Shikanai et al 1998).

It has been reported that a variety of mutants can be isolated by screening for high chlorophyll fluorescence (Miles 1980, Barkan et al. 1986, Meurer et al. 1996, Shikanai et al. 1998). Shikanai et al. (1999) categolized these hcf mutants into three groups after quenching analysis of chlorophyll fluorescence, that is, (1) photochemical quenching mutants having direct impairment in proteins participating in photosynthetic electron transport or the Calvin cycle (Meurer et al. 1996, Dinkins et al. 1997), (2) non-photochemical quenching mutants, in which regulatory machinary of electron transport was abnormal (Niyogi et al. 1998, Niyogi 1999) and (3) mutants with high level of the minimum yield of chlorophyll fluorescence (Fo), possibly including mutation in oxdizing side of PS II, photoinhibition machinary or plastid gene expression.

In this study, one of high Fo mutants of Arabidopsis 2627-2, mutagenized by T-DNA insertion, has been characterized.

Materials and methods

Plant growth conditions

Procedure of screening for high chlorophyll fluorescence seedlings using an image analyzing system was described by Shikanai et al. (1998). A high Fo mutant mutagenized by T-DNA insertion (ecotype Wassilewskija) was used in this study. Plants grew on soil in a growth

chamber at 23 ºC under low light (30 µmol $m^{-2}s^{-1}$, 10 h light / 14 h dark cycles), fertilized with HIPONeX every other day.

Chlorophyll fluorescence analysis

Chlorophyll fluorescence was measured by PAM 2000 portable chlorophyll fluorometer (Walz, Effeltrich, Germany). The minimum chlorophyll fluorescence yield (Fo) was measured under measuring light (650 nm) with very low intensity. Steady state level of fluorescence (F) under actinic white light was also recorded. To estimate the maximum fluorescence yield (Fm and Fm'), a saturating pulse of white light (2,500 µmol quanta $m^{-2}s^{-1}$ for 1s) was applied.

Southern analysis

Genomic DNA from Arabidopsis 2627-2 plants was prepared with Nucleon PhytoPure plant DNA Extraction Kit (Amersham). Southern analysis using *Nde* I and *Bst* 1107I digestions was done after Ponce et al. (1998).

Results and discussion

Genetic analysis

From about 30,000 seedlings mutagenized by T-DNA insertion, 23 putative mutants were isolated for their high chlorophyll fluorescence (Shikanai et al. 1998) A mutant 2627-2 was among 12 mutants that showed high level of the minimum yield of chlorophyll fluorescence (Fo). The high Fo phenotype was found in 16/52 (31%) F2 progenies, showing that the mutation was single and recessive. All F3 progenies from high Fo individual of F2 were kanamycin resistant, suggesting that the high Fo phenotype was linked with T-DNA insertion. The 2627-2 high Fo mutant had pale green leaves and grew very slowly compared with wild type under photoautotrophic growth conditions.

Chlorophyll fluorescence

Quenching analysis of chlorophyll fluorescence (Fig. 1) revealed that 2627-2 had high Fo and light-induced quenching below the level of Fo, which were characteristic in high Fo mutants (Shikanai et al. 1998, 1999) and some high chlorophyll fluorescence (hcf) mutants (Meurer et al. 1996). High Fo phenotype can be derived from diverse types of mutations. For example, the fluorescence induction pattern of this mutant was similar to that of maize mutant impaired in chloroplast ribosomal protein (Schultes et al. 2000) and also to that of Arabidopsis mutant impaired in OEC33 in PSII (Murakami et al, S13-013 in this proceedings). The former mutation would induce general impairment in plastid gene expression, and as a result, photoinhibition of PSII. Thus, the high Fo phenotype is indirectly induced during the growth in the light. In contrast, in the latter mutant, the high Fo phenotype might be directly associated with the mutation. At present, these two types of mutants cannot be distinguished by fluorescence induction patterns, thus, the origin of the mutation in 2627-2 can not be deduced. Application of saturating pulse during illumination induced full reduction level of fluorescence (Fm'). From this and steady state level fluorescence (F), quantum yield of PS II (Φ_{II}) was calculated as (Fm'-F)/Fm' (Genty et al. 1989). The mutant 2627-2 showed lowerΦ_{II} under both high and low irradiation, suggesting photosynthetic electron transport was impaired in this mutant. LowerΦ_{II} under high light than under low light indicating that this mutant was susceptible to photo-oxidative damage.

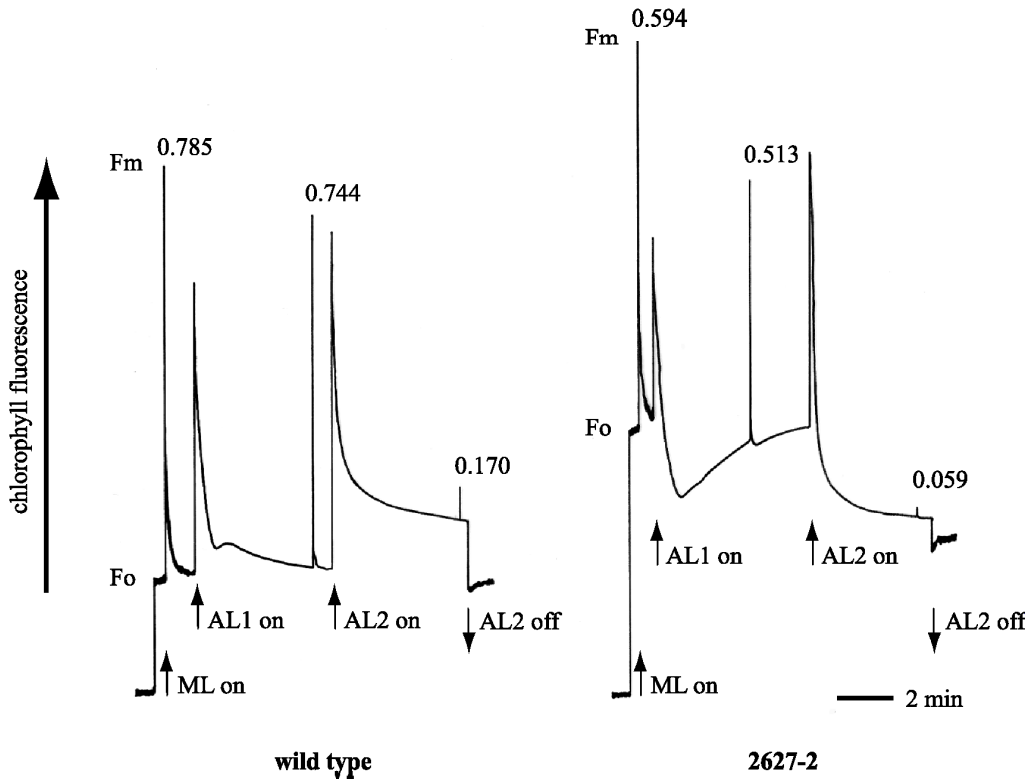

Fig. 1 Quenching analysis of chlorophyll fluorescence

Chlorophyll fluorescence kinetics of dark-adapted wild type and 2627-2 were measured. The leaf was illuminated with measuring light (ML) and actinic light (AL1; 35 μmol quanta $m^{-2}s^{-1}$, AL2; 1250 μmol quanta $m^{-2}s^{-1}$). Values indicate quantum yield of PS II by a saturating pulse of white light (2,500 μmol quanta $m^{-2}s^{-1}$ for 1s). Fo and Fm denote the minimal and maximal chlorophyll fluorescence yield.

Southern blot analysis

Southern analysis using *Nde* I and *Bst* 1107I digestion (Ponce et al. 1998) showed that 2 or 3 copies of T-DNA were tandemly inserted at a single site in 2627-2 genome (not shown). This result supported the previous genetic data and indicated that the insertion of T-DNA conferred the phenotype of this mutant. TAIL-PCR and plasmid rescue are in progress to identify the mutated gene.

Acknowledgement

This work was supported in part by Grants-in-Aid for Scientific Research on Priority Areas (No.09274101, 09274103) from the Ministry of Education, Science, Culture and Sports, Japan (to F.S.) and a grant JSPS-RFTF9616001 (to F.S.).

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