SCREENING FOR MUTANTS DEFICIENT IN STATE TRANSITIONS USING TIME-RESOLVED IMAGING SPECTROSCOPY OF CHLOROPHYLL FLUORESCENCE

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1. Introduction

In green plants and algae, the photosystem II antenna, (light-harvesting complex II, LHC II), has a mobile sub-population that interacts with photosystem II in its non-phosphorylated form but which moves to photosystem I upon phosphorylation. This reversible adaptation changes the distribution of light energy between the two photosystems, allowing equal rates of electron transport during periods where the light conditions favour either photosystem I or photosystem II. If dark-adapted cells, or a single plant leaf, are illuminated with light at 650 nm (absorbed by photosystem II), after an initial maximum fluorescence falls back as light is gradually redistributed from the light-saturated photosystem II to the light-limited photosystem I. This adaptation state to photosystem II light is known as 'state 2'. Addition of light at 700 nm (absorbed by photosystem I) causes a further decrease in photosystem II fluorescence followed by a rise as energy is redistributed to photosystem II. This adaptation state to photosystem I light is known as 'state 1'. For reviews see [1-2].

In order to investigate the role of LHCII in state transitions, and possibly identify the kinase or phosphatase that act upon it, we are currently using time-resolved fluorescence imaging (TRIS) to screen the Feldman population of TDNA tagged *Arabidopsis* plants [3] for mutants that show altered state 1 - state 2 transitions.

2. Procedure

2.1 TRIS

The techniques is based loosely on the procedure of Fenton and Crofts [4], but with the ability to store up to 1,000 images in RAM, and with a camera sensitive enough to permit fluorescence to be defined by a narrow band-pass interference filter, thereby allowing the possibility of additional illumination with light 1. The highly sensitive camera (red-extended ISIS, Intensified CCD, Photonic Science Ltd, East Sussex, UK), has an enhanced red spectral response exhibiting maximal sensitivity, 65 mA/W, at 625 nm. The image acquisition and processing software packages (IonVision and LundVision) were developed by ImproVision (Image Processing and Vision Company Ltd, Barclays Venture Centre, University of Warwick Science Park, Sir William Lyons Road, Coventry CV4 7EZ, UK). Improvision also supplied items of hardware and are able to supply commercially a complete TRIS system of the kind described here. A full description of the technique is submitted for publication [5]. The kinetic data descibed here, and models for the molecular mechanisms involved, will be made available as animated sequences at the URL:

gopher://classic.placebio.lu.se/11ftp:VCB classic HD:

2.2 *Screening procedure*

(1) Grow plants for 14 days in soil (50 µmol m^{-2} sec^{-1} light, 16 hours light/8 hours dark, 20 °C) on 50 mm diameter Petri dishes (these match the area of uniform illumination in the sample chamber); about 30 plants can be screened per plate.
(2) Dark-adapt for 10 minutes.
(3) Illuminate with photosystem II light (light passed through a blue-green Corning CS 4-96 filter) for 6 minutes.
(4) Additionally illuminate with photosystem I light (709 nm) for 2 minutes.
(5) Switch off the photosystem I light and continue the run for a further 2 minutes.
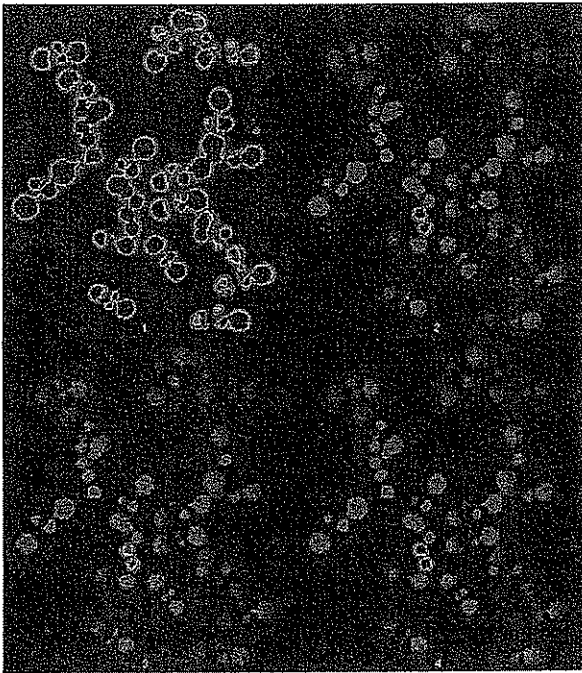
3. Results

Figure 1

Figure 1 shows a selection of 4 from the series of 300 video images produced during one screening. Upon first illumination with photosystem II light (panel 1) there is a large increase in fluorescence; after 6 minutes of continued exposure this has declined to a steady state level (panel 2). Addition of photosystem I light causes a characteristic slight fall in fluorescence (panel 3), reversed upon removal (panel 4).

The image acquisition and processing allows each of the individual plants in the field of view to be delineated as a "region of interest (ROI)" by drawing with the mouse. The fluorescence levels in each ROI can then be calculated from the start to the end of the screen producing data that can be used in a spreadsheet program to create graphs of time versus relative fluorescence.
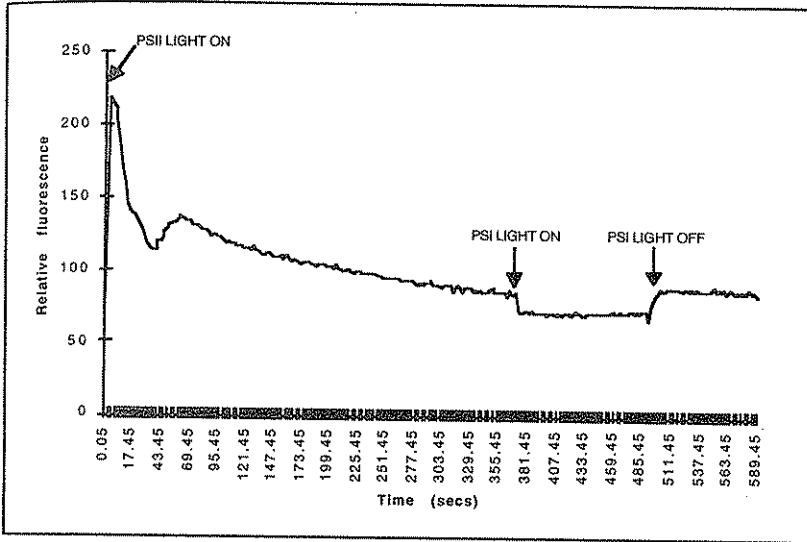

Figure 2

Figure 2 shows the typical fluorescence profile of a wild-type *Arabidopsis* plant. Exposure to photosystem II light causes a rapid rise in fluorescence up to a maximum (F_p) as photosystem II becomes quickly saturated followed by a decline as the primary electron acceptors (Q_A, plastoquinone pool), in a fully oxidised state as a result of the dark treatment, pass electrons on and become reoxidised (photochemical quenching). This is followed by a slower rise up to a secondary maximum and a slow decrease down to a steady state (F_t), mostly the result of non-photochemical quenching mechanisms such as LHC II phosphorylation. Addition of photosystem I light causes further quenching; a very small rise in fluorescence can be observed, as some light energy is redistributed to photosystem II, during the photosystem I light period. Switching off photosystem I light reverses this quenching.

Although this profile (Fig.2) is typical, individual plants do vary, particularly in the extent of the secondary maximum; any kind of stress, e.g. water stress, can dramatically alter the profile and cause a partial or total reduction in the fluorescence quenching by photosystem I light. The latter is also dependent on the age of the plant.

About 5000 TDNA-tagged lines from the Feldman collection have been obtained from the Nottingham Seed Centre, U.K. in pools of 100 lines. Sixteen of these pools have been screened (at least 1000 plants per pool screened) and several mutant fluorescence phenotypes obtained. All putative mutant plants are taken to the next generation to ensure that all the progeny exhibit the phenotype and that it is not simply a stress effect.

Four mutants (3 isolated from the same pool) have been found that show a rise in fluorescence over the photosystem I light period. In this respect they resemble very young plants but they still show this phenotype at age 3 weeks. They have no other visible phenotype.

Two individuals have been isolated from separate pools that show a larger than normal fluorescence drop in response to photosystem I light. One does have a visible phenotype, appearing slightly paler than wild-type. Another, otherwise wild-type, plant shows no discernible secondary maximum and a reduced photosystem I light fluorescence drop (results not shown).

4. Discussion

The technique of "time resolved imaging spectroscopy (TRIS)" has been successfully used to screen for new classes of *Arabidopsis* mutants based solely on their fluorescence profiles in response to altered light conditions. Screening of the Feldman collection of TDNA-tagged lines is continuing and the mutants isolated from the first 1600 lines crossed to wild-type to generate heterozygotes that will be allowed to self to see whether the TDNA is segregating with the phenotype. Seed from each of the mutants is also being bulked up so that they can be analysed biochemically, particularly to determine whether any show altered phosphorylation of LHC II.

TRIS is a powerful technique that can analyse large numbers of plants growing *in vivo* in a relatively short time to allow for the isolation of many new mutants that appear to have no visible phenotype. The wavelength of the incident light and of the light detected by the camera can be easily altered allowing fluorescence from compounds other than chlorophyll to be investigated. The camera is also sensitive enough to be used to *in vivo* image luciferase bioluminescence from the new family of luciferase reporter genes.

Acknowledgements

References

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