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Identification and characterisation of state transition mutants in *Arabidopsis thaliana*

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Introduction

The regulatory processes controlling the photosynthetic light reactions can be divided into short- and long-term mechanisms of adaptation which include LHCII state transitions and PSII repair cycle as well as control of transcription and translation rates of photosynthetic genes. In order to compensate changes in light intensity and quality, photosynthetic active organisms react either with activation/deactivation mechanisms or with *de novo* synthesis/ degradation processes. This kind of photoacclimation requires a concerted regulation of the enzymatic reactions together with nuclear and plastidial gene expression (Allen, 1993, 1995). Most of these regulatory systems are based on control mechanisms via redox-active components inside the cell (Allen and Nilson, 1997; Pfannschmidt et al., 1999). Especially plants are exposed to transient short-term changes in light intensity and quality leading to an imbalance between the excitation rate of PS II and PS I. The regulation of absorbed light energy and avoidance of oxidative damage constitute a major problem. However, adaptations to transient changes are achieved by regulation of photochemistry: In order to balance the absorbed energy distribution between PS II and PS I, phosphorylation of the LHC II results in reversible coupling with the photosystem cores in a mechanism called LHC state transitions (Fork and Satoh, 1986; Bennett, 1979; Gal et al., 1997; Allen and Forsberg, 2001). The work presented here focused on the identification of gene products involved in this important regulatory adaptation process. It included the establishment of a systematic screening of a library of ecotype *columbia NB* mutants from *Arabidopsis thaliana* for the isolation of plants defective in state transitions. These plants should then be used for further molecular genetic and biochemical characterisations.

Materials and Methods

Arabidopsis thaliana ecotype columbia NB mutants were obtained from Leehle Seeds, USA. After 18 days of growth plants with defects in state transitions were identified by fluorescence video imaging according to Kruse et al. (1999).

Plants of interest were isolated and their ability of carrying out state transitions was subsequently determined by 77 K fluorescence spectra and by modulated fluorescence measurements using a Perkin Elmer LS50B fluorometer (Kruse et al., 1999).
Plants were incubated either for 8h in darkness (state 1) or for 4h in standard growth light (state 2) followed by a purification of crude thylakoid membranes according to Vener et al. (2001) with minor changes: Isolated thylakoids were resuspended in 25 mM HEPES pH 7,6, 5 mM $MgCl_2$, 330 mM sucrose, 5mM EDTA .

SDS-PAGE and immunoblotting were performed according to Laemmli (1970) and Burnett et al. (1981) in 6M urea gels containing 10% acrylamide using a polyclonal antibody raised against phosphothreonine (Zymed).

Quenching analyses were carried out with a fluoro cam 690M (Photon System Instruments) using standard software. Non-photochemical and photochemical quenching data were calculated according to Haldrup et al. (1999).

Results

2 x 10^4 *Arabidopsis* NB mutants were screened by video imaging for defects in the mechanism of LHC state transitions following a procedure originally introduced for *Chlamydomonas reinhardtii* mutants (Kruse et al.,1999). As a result, two candidates, stmu1 and stmu2, were successfully isolated. Their inability for functional state transitions was subsequently demonstrated by 77K fluorescence emission spectra (Fig.1a) and modulated fluorescence measurements at room temperature (Fig.1b) with leaves pre-adapted under state 2 conditions and state 1 conditions. No decrease of PSII-related fluorescence was observed in the mutants after pre-incubation with PSII light (620nm) in 77k spectra and no time-resolved increase of chlorophyll a fluorescence could be obtained during incubation with PSI light (700nm) at room temperature.

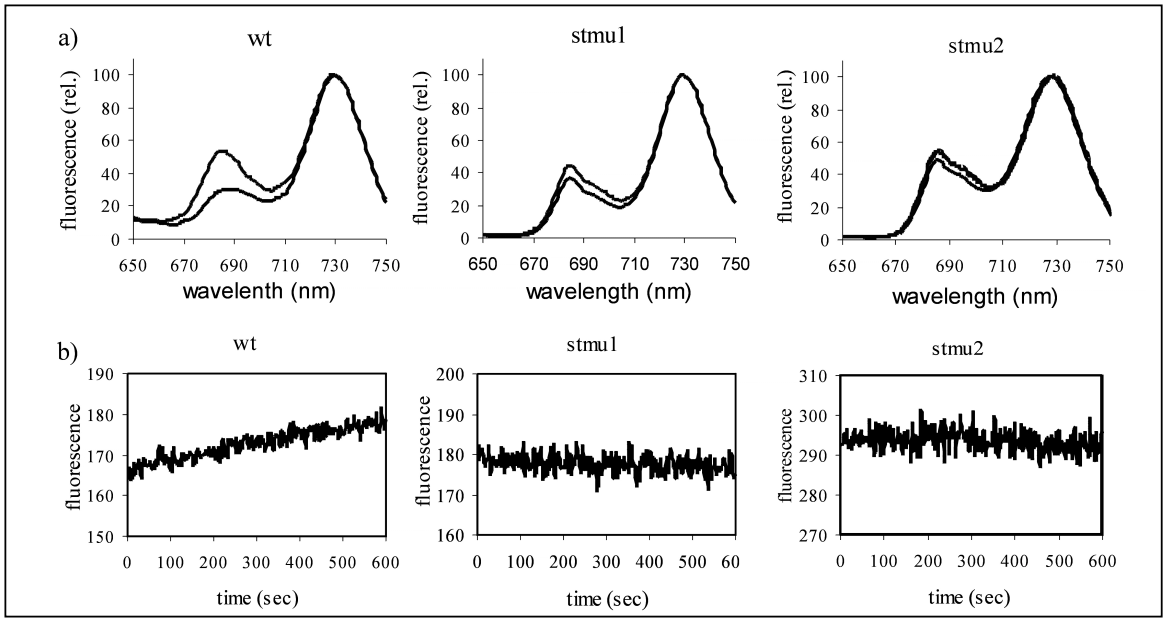

Fig. 1. Evaluation of state transition activity of *Arabidopsis* wild type (wt), stmu1 and stmu2 leaves.

a) 77K fluorescence emission spectra of pre-adapted either to state 2 (grey lines) or to state 1 (black lines)

b) Modulated room temperature fluorescence kinetics. Excitation light to induce state 1 was 700nm.

To assess whether the effects on functional state transitions were related to differences in the levels of LHC protein phosphorylation crude thylakoid preparations from wild type, stmu1 and stmu2 were separated on SDS gels followed by immunoblotting with polyclonal anti-phosphothreonine. Most dominant crossreactions were obtained against the upper of two dominant LHC bands in thylakoid preparations from leaves adapted to state 2 in wt and stmu1 (Fig.2). Thylakoid preparations from leaves adapted to state 1 showed no significant

crossreactions which clearly indicated that under these conditions LHCII is not phosphorylated. In contrast stmu2 showed a significant phosphorylation of the upper LHCII band even under state 1 conditions. It is therefore feasible to suggest that the de-phosphorylation reaction under state 1 conditions is disturbed in stmu2.

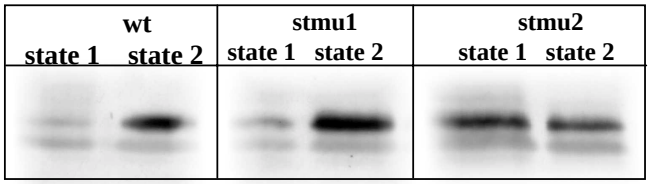

Fig.2. Immunoblots of wt, stmu1 and stmu2 thylakoids isolated from preparations adapted to state 1 (8h darkness) or state 2 (4h growth light) and incubated with a polyclonal antibody (Zymed) raised against phosphothreonine (dilution 1:5000)

To elucidate the functionality of the photosynthetic electron transport chain, PSI- and PSII activity measurements (Φ_{PSI},Φ_{PSII}) as well as photochemical and non-photochemical quenching (q_P, q_N) analyses were carried out. Tab.1 and Fig. 3 summarise the results.

	wt	stmu1	stmu2
1-qP	0,33±0,1	0,75±0,1	0,33±0,1
qN	1,51±0,3	1,22±0,5	1,09±0,1
ΦPSII	0,59±0,1	0,39±0,1	0,58±0,2
ΦPSI	18,39±1,2	23,87±2,8	19,57±3,9

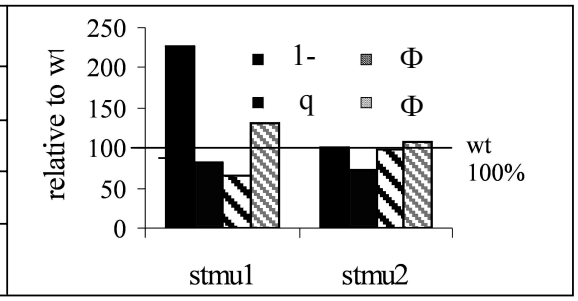

Fig. 3. Photosynthetic parameters of wild type (wt) plants and plants defective in LHC state transitions.

(Table 1):

qP: photochemical quenching (see: Haldrup et al, 1999)

qN: non-photochemical quenching (see: Haldrup et al., 1999)

1-qP: reduction state of the plastoquinone pool

ΦPSII: µmol O_2 μmol^{-1} photons

ΦPSI: µmol O_2 $mgChl^{-1}$ h^{-1}

The quenching data were obtained by video imaging with continuos actinic PSII light illumination (620nm, 250µE m^{-2} s^{-1}) for 20min interrupted by a 800ms super pulse every 60sec. The PSII and PSI activity data indicated that both mutants have fully functional PSII and PSI complexes. However, non-photochemical quenching is reduced by nearly 20% in both mutants whereas the reduced state of the plastoquinone (PQ) pool (1-qp) is generally higher in the mutants. In particular stmu1 shows a 3 times higher reduced state of the PQ pool compared to wt.

Discussion

Two *Arabidopsis* state transition mutants have been successfully identified by fluorescence video imaging. Both mutants grow photoautrophically with no visible damage under standard light conditions. However, further phenotype characterisations demonstrated major differences to wt. Stmu2 is disturbed in a functional phosphorylation/de-phosphorylation reaction of LHCII. The main difference in stmu1 is its unusual high redox state of the PQ pool during illumination with actinic PSII light. Additionally, both mutants are characterised by a reduced capability for non-photochemical quenching (see: Müller et al., 2001). It is of particular note that these features, a higher reduced PQ pool and lower values in photochemical- and non-photochemical quenching, are characteristic for a *Arabidopsis* psaH mutant which was shown to be disturbed in functional state transitions (Lunde et al., 2000). Based on these data it is feasible to suggest that in both mutants genes could be disturbed whose translation products are possibly involved in the regulation processes of functional LHC state transitions. Consequently, PCR-based strategies are introduced to map mutated genes in stmu1 and stmu2 with a set of mapping markers designed for *Arabidopsis thaliana*.

Acknowledgements

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