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Functional aspects of the photosynthetic light reactions in heat stressed *Arabidopsis* deficient in digalactosyl-diacylglycerol

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ABSTRACT

Plants are often submitted, in their natural environment, to various abiotic stresses such as heat stress. However, elevated temperature has a detrimental impact on overall plant growth and development. We have examined the physiological response of the *dgd1-2* and *dgd1-3 Arabidopsis* mutants lacking 30–40% of digalactosyl-diacylglycerol (DGDG) exposed to heat constraint. These mutants, which grow similarly to wild type under normal conditions, were previously reported to be defective in basal thermotolerance as measured by cotyledon development. However their functional properties were not described. Chlorophyll fluorescence measurements and absorbance changes at 820 nm were used to monitor photosystem II (PSII) and PSI activity, respectively. It was observed that both mutants have similar photosystem activities with some differences. The mutants were less able to use near saturation light energy and elicited higher rates of cyclic PSI electron flow compare to wild type. *Arabidopsis* leaves exposed to short-term (5 min) mild (40 °C) or strong (44 °C) heat treatment have shown a decline in the operating effective quantum yield of PSII and in the proportion of active PSI reaction centers. However, cyclic PSI electron flow was enhanced. The establishment of the energy-dependent non-photochemical quenching of chlorophyll fluorescence was accelerated but its decline under illumination was inhibited. Furthermore, heat stress affected the process implicated in the redistribution of light excitation energy between the photosystems known as the light state transitions. All the effects of heat stress mentioned above were more intense in the mutant leaves with *dgd1-3* being even more susceptible. The decreased DGDG content of the thylakoid membranes together with other lipid changes are proposed to influence the thermo-sensitivity of the light reactions of photosynthesis towards heat stress.

Introduction

The efficiency of photosynthesis is affected by many types of environmental stress such as high salt concentration, abnormal level of reactive oxygen species (ROS), and unusually high and low temperature (Allakhverdiev et al., 2001; Allakhverdiev and Murata, 2004). According to the literature, there are two principal modes of stress-induced impairment of photosynthesis: first, a direct damage induced by the stress factor and second, inhibition of de novo protein synthesis by resulting ROS (Allakhverdiev et al., 2008). Heat stress is one of the main abiotic stresses that limit plant biomass production and productivity, especially, in tropical and subtropical countries (Boyer, 1982). There are, at least, three major stress-sensitive sites in the photosynthetic machinery: the photosystems, mainly photosystem II (PSII) with its oxygen-evolving complex (OEC), the ATP generating system, and the carbon assimilation processes (Carpentier, 1999; Murata et al., 2007; Mohanty et al., 2007). PSII is the critical site of damage by low and high temperatures together with various stress factors such as drought, salinity, and UV radiation (Berry and Björkman, 1980; Allakhverdiev et al., 2008).

Heat constraint induces significant changes in the composition of chloroplast membrane lipids and proteins that results in structural modifications of the thylakoid membrane (Carpentier, 1999; Santarius, 1980). Alteration in lipids could affect selective stress signalling either through global effects on the physical state of the membrane, or via specific interactions of lipids with proteins (Vígh et al., 2007 and references therein). Growth at low temperature causes increases in fatty acid unsaturation and changes in

Abbreviations: AL, actinic light; Chl, chlorophyll; DAG, diacylglycerol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DGDG, digalactosyl-diacylglycerol; ER, endoplasmic reticulum; ETR, electron transport rate; FR, far-red light; MGDG, monogalactosyl diacylglycerol; ML, measuring light; MV, methyl viologen; NPQ, non-photochemical quenching; PAM, pulse amplitude modulated fluorimeter; PC, phosphatidylcholine; PG, phosphatidylglycerol; PQ, plastoquinone; PSII, photosystems II; PSI, photosystems I; P680, primary electron donor of photosystem II; P700, primary electron donor of photosystem I; ROS, reactive oxygen species; SQDG, sulfoquinovosyl-diacylglycerol.

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the proportions of lipid classes (Hazel, 1995; Vígh et al., 1998). It is reported by Los and Murata (2004 and references therein) that the membrane fluidity decreases with a decrease in temperature whereas high temperatures cause the fluidization of the membrane. Indeed, a previous study made on *Synechocystis* (Horváth et al., 1998) demonstrated that the membrane fluidity was adjusted by temperature acclimation. These changes, along with the direct inactivation of photosystems, greatly affect the photochemical activity of chloroplasts (Ilik et al., 2003). It is believed that thermal denaturation of PSII is directly related to the major changes in the lipid phase of the thylakoid membrane that occur at high temperature (Berry and Björkman, 1980; Quinn and Williams, 1985; Yordanov et al., 1986; Larkindale et al., 2005). Presumably, one of the main consequences of these lipid changes is a destabilization of lipid–protein interactions, thus perturbating the organisation and function of PSII. More precisely, the oxygen-evolving/water splitting complex is disrupted, with functional manganese ions and extrinsic proteins being released (Nash et al., 1985a,b; Enami et al., 1994), and the peripheral light-harvesting system separates from the PSII complexes (Armond et al., 1980; Gounaris et al., 1983) with the reduced size PSII migrating from the appressed regions of the thylakoid membranes to the non-appressed regions (Sundby et al., 1986). Hence, Kreslavski et al. (2008) have shown in wheat that exposure to short-term heat stress (42 °C) induces a progressive destacking of the thylakoid membranes.

Polar lipid composition strongly influences the structure and efficiency of the thylakoid membrane via specific lipid–protein interaction and the dynamic properties of the lipid bilayer (Webb and Green, 1991; Vígh et al., 2007). The unique lipid composition of thylakoid membranes is dominated by the two galactolipid head groups monogalactosyl-diacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG) comprising approximately 50% and 20% of the total thylakoid acyl lipid content, respectively. The remaining lipids include phosphatidylglycerol (PG) and sulfoquinovosyldiacylglycerol (Webb and Green, 1991). Suss and Yordanov (1986) demonstrated that the thermotolerance of photosynthetic light reactions is favoured by an increased ratio of DGDG to MGDG and with an increase in the saturation level of DGDG. Indeed, the digalactosyl-diacylglycerol level and the ratio of DGDG to MGDG are higher in barley etioplasts grown at 30 °C than in those grown at 20 °C (Di Baccio et al., 2002).

In the present study, our investigations focus on the determination of the response of *dgd1-2* and *dgd1-3 Arabidopsis* mutants deficient in DGDG (Chen et al., 2006) to short-term heat stress compare to wild type (WT). These mutants grow similarly to WT under normal conditions but were reported to be defective in basal thermotolerance as measured by cotyledon development. However, their functional properties were not described previously. We have found that the modified lipid content in the thylakoid membranes of the mutants caused an enhanced thermo-sensitivity of the photosynthetic functions. Surprisingly, the *dgd1-3* mutant, who shares a similar mutation and DGDG content with *dgd1-2*, was more sensitive. The thermo-sensitivity of the photosynthetic apparatus thus depends on the decreased DGDG content of the thylakoid membranes whereas the stronger heat-induced damages in *dgd1-3* are proposed to be due to other consequent changes in thylakoid membrane lipids that differed in the two mutants.

Materials and methods

Plant growth conditions

Seeds of *Arabidopsis* WT and mutants (*dgd1-2* and *dgd1-3*) were sown into soil (Promix, Premier Horticulture Ltee/Ltd, Rivières-du-Loup, Canada) and were pre-treated at 4 °C for 3–4 days to promote germination. They were then transferred to the growth chamber at 22/18 °C (day/night) with a 16-h photoperiod at a photon flux density of 100 μmol m^{-2} s^{-1} and 75% humidity. Water and nutrients were routinely added to prevent any growth limitation. Mature, healthy and non-senescent rosette leaves from 6 to 7 week-old plants were used for heat stress treatments.

Chlorophyll fluorescence

Chlorophyll (Chl) fluorescence from dark adapted (2 h) detached leaves was measured with a pulse amplitude modulated fluorometer (PAM 101–103; Walz, Germany) as described in Schreiber et al. (1988). State 2 was induced using the beam of the KL 1500 projector filtered through a Schott BG-39 (80% transmittance around 500 nm and no transmittance beyond 600 nm, 100–120 μmol m^{-2} s^{-1}). A beam of far-red light (FR) (23 μmol m^{-2} s^{-1}) used to induce state l transition was delivered from a Filtre-Lite light source (Microview, Thornhill, ON, Canada) in combination with a RG-9 filter (Schott, Mainz, Germany). Under steady-state fluorescence, a saturating white pulse was applied to measure the yields of F_{m1} and F_{m2}, respectively. The relative changes in state transition, ST, were determined from $ST = [(F_{I'} - F_I) - (F_{II'} - F_{II})]/F_{I'} - F_I$ (Lunde et al., 2000), F_I and F_{II} represent the fluorescence in the presence of FR-light in state 1 and 2, respectively and $F_{I'}$ and $F_{II'}$ correspond to the fluorescence in the absence of FR-light in state 1 and 2, respectively.

Measurements of PSII parameters

Non-photochemical quenching (NPQ) of the absorbed energy was measured with a dual-modulated Fluorometer, FL-3500 equipped with Leaf-Clip (Photon Systems Instruments, Brno, Czech Republic). Fluorescence was evaluated following low-intensity measuring flashes of 4 μs (λ = 455 nm). Leaves were exposed for 600 s of actinic light (AL) ranging from 20 to 650 μmol m^{-2} s^{-1} provided by light-emitting diodes (LEDs) at 625 nm. This was followed by a dark-period of 300 s. Strong light pulses (1500 μmol m^{-2} s^{-1}, λ = 625 nm) of 1-s were given periodically to assess the maximal fluorescence level during dark–light–dark transitions. Minimum fluorescence (F_o) was obtained with open PSII centers following excitation by a weak measuring light. A saturating pulse of white light (900 ms, 1500 μmol m^{-2} s^{-1}) was applied to determine the maximum fluorescence with closed PSII centers in dark adapted state (F_m) and during AL illumination (F'_m). The steady-state fluorescence level (F_s) was recorded during AL illumination (125 μmol m^{-2} s^{-1}). $F_v/F_m = (F_m - F_o)/F_m$ represents the maximum quantum yield of PS II. NPQ and electron transport rate (ETR) were calculated as described by Maxwell and Johnson (2000). The quantum yield was calculated as $\Phi_{PSII} = (F'_m - F_s)/F'_m$ (Genty et al., 1989; Maxwell and Johnson, 2000). Nonphotochemical quenching was obtained from Chl fluorescence data as $NPQ = (F_m - F'_m)/F'_m$. ETR relative to PSII was calculated as Φ_{PSII} × light intensity (μmol m^{-2} s^{-1}). The error bars were obtained from the standard errors values, which were derived from the standard deviation calculation.

Heat treatment

The leaves were floated on tap water pre-warmed to respective temperatures in a controlled water-bath for 5 min before initiating the temperature treatments, the control measurements were made. After the temperature treatments in the dark, the leaves were kept at room temperature between moistured tissue paper and used for the analysis of Chl fluorescence and leaf absorbance changes at 820 nm.