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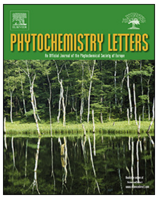

The impact of light intensity on metabolomic profile of *Arabidopsis thaliana* wild type and *reticulata* mutant by NMR spectroscopy

Tahereh Jafari[a,*], Moona Rahikainen[b], Elina Puljula[a], Jari Sinkkonen[a], Saijaliisa Kangasjärvi[b]

[a] *Instrument Centre, Department of Chemistry, University of Turku, FI-20014 Turku, Finland*
[b] *Molecular Plant Biology, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland*

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ABSTRACT

Light acclimation involves biochemical, metabolic and developmental adjustments that allow plants to cope with a vast range of growth environments. *Arabidopsis thaliana* mutants with photoperiod-dependent defects in leaf development and metabolism have been instrumental in deciphering the interlinked regulatory networks in plants. The *reticulata* (*re*) mutant displays dark green veins and pale green mesophyll tissues when grown under long day conditions. RE is a chloroplast envelope membrane protein of unknown function and is required for accurate primary metabolism and leaf development under long photoperiod, while its functional significance under short photoperiods has remained poorly understood. In the present study we assessed whether RE impacts primary metabolism or leaf development when *Arabidopsis* plants acclimate to different light intensities under short photoperiod. We show that growth under short day conditions annuls the metabolic and developmental defects of *re* mutants, suggesting that RE does not significantly modulate leaf development or primary metabolism under short photoperiod. Based on proton nuclear magnetic resonance spectroscopy (^{1}H NMR) and statistical analysis, however, the metabolite profiles of differentially light-acclimated short-day-grown plants differ with respect to sugars (glucose, fructose and sucrose), TCA cycle intermediates (fumaric, malic, citric and succinic acids) and fatty acids, which become more abundant under high light. Moreover, in contrast to isoleucine, leucine, valine, threonine, serine, tyrosine and phenylalanine, which show increased abundance in high-light-acclimated plants, the contents of alanine, glutamine, glutamic acid and aspartic acid are higher when plants grow under normal growth light. These findings indicate that NMR can detect high-light-induced metabolic adjustments that arise upon plant acclimation to light stress.

1. Introduction

Plants respond to prevailing light conditions by eliciting metabolic and developmental adjustments that allow completion of their life cycle under a vast range of growth environments. Light is a key determinant of leaf development and optimizes the formation of photosynthetically active tissues, where the light-driven redox reactions occurring in chloroplasts convert solar energy into chemical form. Besides yielding photosynthetic end products, chloroplasts contribute to the biosynthesis of fatty acids, amino acids, vitamins, hormones and specialized secondary metabolites, which not only provide building blocks for the developing tissues, but also defend the plant against environmental stress agents. Imbalances in the light-driven redox chemistry, on the other hand, promote transient generation of reactive oxygen species (ROS), which serve important signaling functions under environmental challenges.

Appropriate development of leaf anatomy is critical for photosynthetic light harvesting, gas exchange and metabolic activities (González-Bayón et al., 2006; Pérez-Pérez et al., 2013) and leaf vasculature, especially the bundle sheath cells with specified metabolic properties, appear to mediate key functions in the underlying regulatory processes (Mullineaux et al., 2006; Yu et al., 2007; Cheng et al., 2006; Fryer et al., 2003; Kangasjärvi et al., 2009). In *Arabidopsis* leaves, vascular tissues, especially the photosynthetic bundle sheath cells, display unique characteristics of ROS metabolism and accumulate hydrogen peroxide (H_2O_2) to elicit acclimation processes in high-light-illuminated leaves (Fryer et al., 2003).

In addition, metabolic reprogramming is a key element that allows plants to cope with light stress. Studies on *Arabidopsis* have illustrated high-light-induced alterations in carbon metabolism, presumably caused by altered photosynthetic activity and the associated activation of protective mechanisms (Jänkänpää et al., 2012; Wulff-Zottele et al., 2010).

Arabidopsis mutants have also been instrumental in revealing

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[*] Corresponding author.
E-mail address: tahereh.jafari@utu.fi (T. Jafari).

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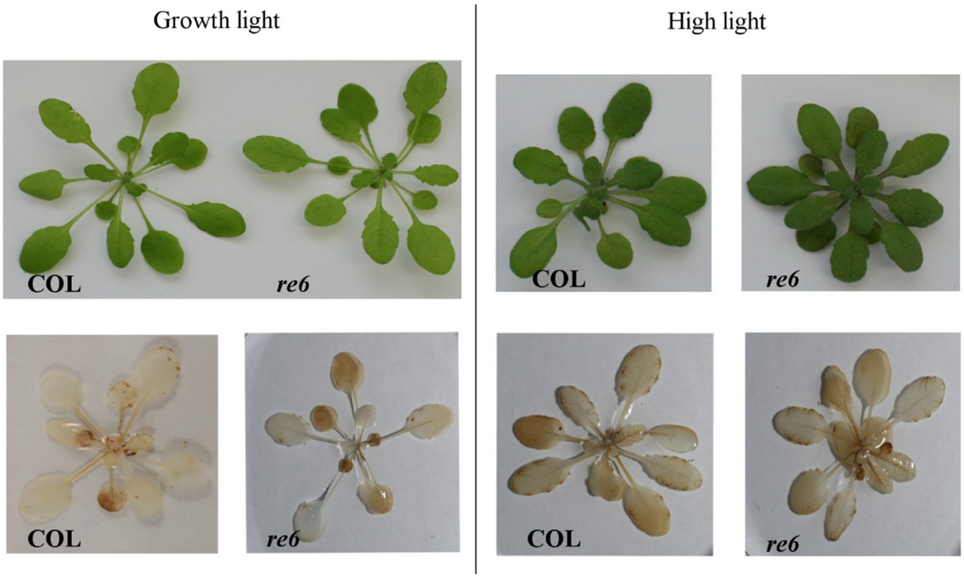

Fig. 1. (Up) *Arabidopsis* wild type COL and *reticulata* (*re6*) mutant grown under short day, 130 μmol photons $m^{-2}s^{-1}$ (growth light) or 500 μmol photons $m^{-2}s^{-1}$ (high light). **(Below)** DAB-staining of H_2O_2 included *Arabidopsis* wild type (COL) and *reticulata* (*re6*) under short day and 130 μmol photons $m^{-2}s^{-1}$; Wild type (COL) and *re* under short day and 600 μmol photons $m^{-2}s^{-1}$.

metabolic and regulatory cross-talk in plants. The *reticulata* (*re*) mutant, also identified as *lower cell density1* (*lcd1*; Barth and Conklin, 2003) and *radical-induced cell death2* (*rcd2*; Overmyer et al., 2008), shows accumulation of H_2O_2 in leaf veins and displays a reticulate phenotype with pale green mesophyll tissues when grown under long day conditions (González-Bayón et al., 2006; Li et al., 1995; Mollá-Morales et al., 2011). The *re* mutants display fewer mesophyll cells and smaller plastid size in the interveinal leaf regions as compared to wild type plants (Lundquist et al., 2014). The allelic *lcd1-1* mutant was identified in a screen for ozone (O_3) sensitivity, which was accompanied by bleaching of chlorophyll and accumulation of H_2O_2 along the vasculature (Barth and Conklin, 2003).

RE belongs to a family of six chloroplast envelope membrane proteins of unknown function and localizes primarily to bundle sheath cell chloroplasts along the vasculature (Pérez-Pérez et al., 2013). Extensive genetic and phenotypic analysis, together with transcriptomic and metabolomic profiling, revealed that RE family members are required to maintain amino acid homeostasis and accurate leaf development through partially over-lapping functions when plants grow under long day conditions (Pérez-Pérez et al., 2013). Extensive genetic and phenotypic analysis, together with transcriptomic and metabolomic profiling, revealed that RE family members are required to maintain amino acid homeostasis and accurate leaf development through partially overlapping functions. Comparison of *Arabidopsis re* and *reticulata-related* (*rer*) mutants demonstrated that leaf reticulation associated with changes in ROS homeostasis and amino acid metabolism when plants grew under long day conditions (Pérez-Pérez et al., 2013). The nature of these molecular mechanisms, however, still remains unresolved.

While the importance of *re* on metabolic and regulatory interactions in long day conditions is relatively well established, its physiological significance under short day conditions in different light intensities has remained poorly understood.

The chemical composition and metabolites were measured by nuclear magnetic resonance spectroscopy (NMR). NMR is a technique based on exciting certain nuclei in a given sample with radio frequency pulse and measuring those frequency signals which have been emitted by the nuclei (Günther, 2013). Proton nuclear magnetic resonance spectroscopy (^1H NMR) and statistical analysis can be used for exploring a large number of metabolites in the targeted object (Sekiyama et al., 2011).

Here we assessed how short day and growth light intensity impact morphological and metabolomic characteristics of *Arabidopsis* wild type and *re* mutant plants and analyzed their chemical composition and metabolites by nuclear magnetic resonance spectroscopy (NMR). We show that growth in short day conditions alleviates the phenotypical characteristics of *re* mutants, irrespective of the growth light intensity, and also the metabolite profiles of wild type and *re* mutants are alike. The metabolite profiles of differentially light-acclimated plants, however, display distinct differences, as evidenced by a number of primary metabolites and fatty acids that became more abundant in high-light-grown wild type and *re* plants as compared to plants grown under normal growth light. We conclude that exposure of plants to high light intensities triggers metabolic adjustments, presumably to enhance plant stress tolerance under challenging environmental cues. Under long photoperiods, RE is an essential component determining primary metabolism and leaf development.

Under short photoperiod, however, the functionality of RE is not critical in metabolic and developmental regulation in *Arabidopsis*.

2. Results and discussion

2.1. Phenotypes of wild type and reticulata plants under short day conditions

Phenotypic comparison of wild type and *re6* distinguished no reticulation in the rosette leaves of *re6* under short day and high light conditions (Fig. 1) and similar observations were made for the allelic *re4* and *re8* mutants (Fig. S1, Supplementary data). In line with the visual phenotypes, quantification of chlorophyll revealed no major differences between wild type and *re* when grown under growth light and high light (Table S1). Hence, growth under short photoperiod annuls the developmental defect of *re* mutants regardless of the growth light intensity.

Next we explored the pattern of ROS accumulation in wild type and *re* mutant leaves with diaminobenzidine (DAB), which reacts with H_2O_2 causing appearance of a brown colored precipitation (Vanacker et al., 2000). DAB-staining of wild type and *re8* under short day and growth light revealed no detectable H_2O_2 accumulation in either of the genotypes (Fig. 1). Also under short day and high light, similar DAB-staining activity was observed between *re* and wild type (Fig. 1). For comparison, DAB-staining of wild type Col, *re4* and *re6* under long day and short day growth light and high light is presented in supplementary information (Fig. S2).