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# Genome-wide association studies of Arabidopsis dark-induced senescence reveals signatures of autophagy in metabolic reprogramming

Feng Zhu [a,b], Saleh Alseekh [b,c], Weiwei Wen [a,b], Yunjiang Cheng [a,b], and Alisdair R. Fernie [b,c]

[a]National R&d Center for Citrus Preservation, Key Laboratory of Horticultural Plant Biology, Ministry of Education, Huazhong Agricultural University, Wuhan, China; [b]Department Willmitzer, Max-Planck-Institut Für Molekulare Pflanzenphysiologie, Potsdam-Golm, Germany; [c]Department Plant metabolomics, Center of Plant Systems Biology and Biotechnology, Plovdiv, Bulgaria

**ABSTRACT**

Macroautophagy/autophagy is a conserved mechanism responsible for the degradation of unnecessary or dysfunctional components and recycling of the nutrients they contain in order to promote cellular or organismal longevity. In plants photosynthesis is massively impaired under extended darkness stress and the transition to heterotrophic metabolism results in carbon and nitrogen starvation which induces metabolic and autophagic shifts to recycle nutrients for plant survival. The majority of research concerning dark-induced senescence focuses on single genes or pathways, and the global characterization of primary and lipid metabolites and autophagy remains limited. To address these aspects we recently developed a time-resolved genome-wide association-based approach to analyze these shifts following 0 d, 3 d and 6 d of darkness. Six patterns of metabolic shifts and 215 associations with enzymes, transcriptional regulators and autophagy genes (such as *AT2G31260/ATG9, AT4G16520/ATG8F, AT5G45900/ATG7* and *AT2G05630/ATG8D*) were identified. Furthermore detailed characterization of candidate genes further demonstrated that the metabolic and autophagic shifts in response to dark-induced senescence is under tightly coordinated genetic regulation.

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Owing to their immotile nature characteristics, plants cannot escape from the stresses prevailing in their environment. One such stress is extended darkness, which generally results in nutrition starvation and cellular damage. The primary effect of darkness is the cessation of photosynthesis and the shift of plant metabolism from an autotrophic to a heterotrophic status leading to the recycling and remobilization of carbon (C) and nitrogen (N) sources to ensure their efficient usage. Moreover, the universal degradation mechanism of autophagy also plays important roles in nutrition recycling via the degradation of unwanted and dysfunctional cellular constituents. Furthermore, the comprehensive analysis of the transcriptome and metabolome of Arabidopsis autophagy-deficient mutants further confirms its crucial role in the metabolic shifts that characterize dark-induced senescence. However, although the high-throughput transcriptome data have supplied considerable insight into the transcriptional response to dark-induced senescence, our knowledge concerning the interactions of large-scale metabolic shifts of the recycling of nutrients and autophagy remains fragmentary.

To analyze the dynamic genetically encoded landscape of the metabolic shift and autophagy in dark-induced senescence, we recently analyzed the primary metabolite and lipid content of the 252 accessions of *Arabidopsis thaliana* natural diversity panel under extended darkness for a period of 0, 3 and 6 d [1]. As the patterns in shifts in metabolite levels during the darkness treatment can reflect the function of these metabolites during this stress, the average value of each metabolite among all accessions in the three time points were calculated and six patterns of metabolite shifts were identified. Given that glycolysis and the TCA cycle play essential roles in energy metabolism, it was not surprising that metabolites related to these pathways such as sugars, sugar alcohols, and organic acids were significantly decreased on the initiation of darkness, and that changes in these metabolites are early indicators for the onset of senescence. Besides the recycling of C, during darkness plants also reuse N produced by the degradation process of amino acids through aminotransferase reactions and (re)assimilation by the glutamine synthetase-glutamate synthase cycle. The high N recycling rate under darkness was confirmed by the continued increase of remobilization metabolites such as glutamine, glutamate, asparagine, ornithine and arginine. Moreover, the alternative respiratory substrates, branched-chain amino acids (leucine, isoleucine, and valine), tyrosine, and lysine content were initially significantly increased during the early darkness treatment owing to protein degradation and then dramatically decreased in the extended darkness treatment due to degradation to supply energy metabolites. As such, their response to dark stress can be regarded as bi-phasic.

Next, given that the variation of metabolite levels is under the regulation of genetic variation, we used a metabolite genome-wide association study (mGWAS) to identify the association between the specific genetic variations with certain metabolite levels across the population, by performing mGWAS analysis based on the metabolite content and

**CONTACT** Alisdair R. Fernie fernie@mpimp-golm.mpg.de Max-Planck-Institut Für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, Potsdam-Golm 14476, Germany

1.2 M imputed and single nucleotide polymorphism (SNP) information of the Arabidopsis accessions for each individual time point (0, 3 and 6 d) as well as the difference in metabolite levels between the different time points (0–3, 3–6 and 0–6 d) to identify the novel genes involved in the metabolic and autophagic shifts that occur during the extended darkness. In the mGWAS results, 215 associations were found between the metabolites and variation of SNPs, with genes associated with metabolite degradation and transport or transcriptional regulation being identified. Interestingly, the SNPs near several autophagy genes including *ATG9, ATG8F, ATG7* and *ATG8D* exhibit strong association with triacylglycerides 52:3, benzoic acid, lysine and GABA, respectively, confirming the important role of autophagy in lipid and primary metabolites reusage.

In summary, our study presented a global model, which has allowed us to dramatically refine our knowledge of the metabolic and autophagic shifts that occur during dark-induced senescence in plants. When the kinetics of the associations of autophagy-related genes with metabolite levels are considered one, that of *ATG9*, appears only in the 3 d and 0–3 d datasets, while *ATG8F* appears only in the 6 d and 0–6 d datasets, suggesting that the influence of these proteins occurs at different time-points of the senescence process. The associations of *ATG7* and *ATG8D* with metabolites were only observed at time zero, i.e., before the onset of senescence, and are thus more likely to be involved in housekeeping roles rather than the specific response to the dark stress. To our knowledge this study represents the first GWAS evaluation of dark-induced senescence in plants and provides many new interesting leads linking autophagy with the metabolic shifts which this process induces.

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## Disclosure statement

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## ORCID

Feng Zhu http://orcid.org/0000-0002-9133-0843
Saleh Alseekh http://orcid.org/0000-0003-2067-5235
Weiwei Wen http://orcid.org/0000-0002-3413-4766
Yunjiang Cheng http://orcid.org/0000-0002-9159-9455
Alisdair R. Fernie http://orcid.org/0000-0001-9000-335X

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