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Review of the PhD thesis entitled: "The role of CIA2 and CIL in the regulation of chloroplast biogenesis, photosynthesis, and foliar temperature in *Arabidopsis thaliana*"
by Ms Roshanak Zarrin Ghalami

Significance of the research

The thesis addresses an important and timely problem in plant molecular physiology: how nuclear regulation of chloroplast development affects photosynthetic performance, energy dissipation, and leaf thermal balance. The work starts from the observation that plants are frequently exposed to irradiance exceeding their capacity for photosynthetic CO_2 fixation. Consequently, absorbed energy must be partitioned among photochemistry, non-photochemical quenching (NPQ), and evaporative cooling. When these processes are insufficiently coordinated, excess excitation can contribute to photoinhibition, photooxidative stress and harmful foliar warming.

The thesis focuses on the transcriptional regulators CIA2 and CIL and their role in chloroplast biogenesis and photosynthetic development in *Arabidopsis thaliana*. A particular strength of the rationale is its integrative character: chloroplast development, photosystem assembly, pigment accumulation, NPQ response, transpiration and foliar temperature are appropriately considered as interconnected components of plant energy management system rather than as isolated processes. The thesis also identifies an important knowledge gap concerning the quantitative contribution of photosynthetic energy dissipation to leaf heat balance and the possible relationship between chloroplast developmental status and stomatal regulation.

Aims and Goals

The main objective of the thesis was to determine how CIA2 and CIL transcriptional components coordinate chloroplast development with photosynthetic performance and thermal dissipation of excess absorbed light energy in a model plant, *Arabidopsis thaliana*.

The work combines physiological, biochemical, microscopic and transcriptomic approaches using the Col-0 wild type, cia2, cil, cia2cil double mutant, and complemented lines. The objectives were to establish the role of CIA2/CIL in chloroplast maturation and photosystem II (PSII) assembly; to determine how their absence affects photosynthetic performance and energy partitioning; and to clarify how photochemistry, NPQ and transpiration contribute to whole-leaf temperature.

The central hypothesis was that foliar temperature emerges from the balance among photochemical energy use, NPQ-mediated heat dissipation and evaporative cooling. The specific hypotheses propose that CIA2 and CIL regulate chloroplast development and PSII assembly, and that CIA2/CIL-dependent chloroplast development influences energy partitioning and whole-leaf thermoregulation, potentially through effects on stomatal behaviour.

Description of the thesis

The thesis is organized around three scientific articles supported by a thematically related introduction, description of methodology applied in the work, followed by results and discussion sections.

The first article is a review that synthesises current understanding of absorbed excess light energy, photoinhibition, NPQ, transpiration and plant thermal balance. It identifies knowledge gaps including the need for quantitative calorimetric assessment of NPQ-associated heat production and better understanding of links between chloroplast development and stomatal feedback regulation.

The second article is an experimental study of CIA2 and CIL during de-etiolation. Using mutant and complementation lines together with pigment analyses, chlorophyll fluorescence, molecular assays, immunoblotting and microscopy, the study examines chloroplast development and the establishment of photosynthetic competence. The results show delayed greening, reduced chlorophyll accumulation, smaller and structurally disordered chloroplasts, and delayed accumulation of PSII-associated components in the mutants.

The third article examines the physical and physiological basis of foliar heat emission. Infrared thermography and photo-calorimetry were combined with photosynthetic and stomatal measurements to quantify heat production and to test the roles of photochemistry, NPQ and transpiration. The resulting framework connects molecular control of chloroplast development with whole-leaf thermal physiology.

Methodologically, the thesis is multidisciplinary combining time-resolved de-etiolation experiments, pigment quantification, chlorophyll fluorescence, promoter reporter analysis, immunoblotting, confocal and transmission electron microscopy, RT-qPCR, infrared thermography, photo-calorimetry, as well as gas exchange and stomatal measurements.

Most significant discoveries

1. CIA2 and CIL are important for proper chloroplast maturation. Their disruption delays greening, reduces chlorophyll accumulation and produces smaller, structurally disordered chloroplasts. Complementation substantially restores these phenotypes.

2. CIA2/CIL affect the photosynthetic performance. The work reports delayed accumulation of Lhcb2 and PsbA components of PSII together with altered expression of genes associated with protein import, including TOC159, TOC33 and TIC110, as well as GLK transcriptional regulators and plastid ribosomal components.

3. Illuminated leaves release a substantial fraction of absorbed irradiance as heat. Under the experimental conditions examined, heat emission was approximately 30 ± 7% of absorbed energy. Photo-calorimetric measurements across *Acer*, *Euphorbia* and *Arabidopsis* were in the approximate range of 106.7 – 111 W m^{-2}.

4. Photosynthetic activity and NPQ contribute measurably to foliar thermal balance. Inhibition of PSII electron transport with PSII acceptor side inhibitor DCMU reduced NPQ and leaf warming, with dose-dependent effects on temperature, F_v/F_m ratio and NPQ.

5. Transpiration is a major thermal sink. Restricting transpiration with lanolin increased foliar temperature, supporting the importance of evaporative cooling in maintaining leaf thermal balance.

6. The *cia2cil* double mutant exhibits a distinctive thermal phenotype. It remained cooler than Col-0 wild type and the complemented line under several treatments, while also displaying higher stomatal conductance and lower pigment content. The lower temperature therefore occurs alongside reduced photochemical competence rather than representing an unambiguous improvement in photosynthetic performance.

7. Transcriptomic analysis indicates broader changes in hormonal and stress-related signalling, including pathways associated with jasmonate, ABA, ethylene and auxin, together with changes in pigment and light-response genes. These findings provide a plausible molecular context for altered stomatal and stress responses.

Taken together, the thesis proposes an integrated model in which CIA2/CIL-dependent nuclear regulation controls chloroplast biogenesis and photosynthetic capacity, which in turn affects internal heat production through photochemistry and NPQ. Stomatal conductance and transpiration provide a counterbalancing mechanism for heat removal. The *cia2cil* phenotype illustrates that a cooler leaf is not necessarily a more photosynthetically competent leaf.

Points for further discussion

1. Causality in chloroplast-stomatal communication. The association between CIA2/CIL function, transcriptomic changes in plant hormone pathways and altered stomatal conductance is compelling, but the direct mechanism linking chloroplast developmental status to stomatal regulation remains to be established. Future experiments targeting guard-cell-specific processes, ROS/redox signalling or hormonal perturbations could strengthen this part of the model. I would be interested in the Candidate's opinion on this.

2. Interpretation of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) experiments. DCMU is an effective pharmacological inhibitor of PSII electron transport, NPQ and associated processes, but its effects are not restricted to NPQ alone. Consequently, reductions in leaf temperature after DCMU treatment should be interpreted as evidence for a contribution of PSII-dependent energy dissipation rather than as an exclusive measure of NPQ-derived heat. Genetic manipulation of specific NPQ components could provide a more selective test. Have such tests been undertaken?

3. Environmental interpretation of photo-calorimetry. Photo-calorimetric measurements provide valuable quantitative evidence, but submerged leaves in a controlled calorimetric environment differ from intact leaves in air, where convection, long-wave radiation and transpiration also contribute to energy balance. The reported approximately 30 ± 7% heat fraction should therefore be regarded as condition-specific rather than universal.

4. Attribution of heat to individual processes. NPQ is primarily an operational fluorescence measure, whereas calorimetry measures heat more directly. Combining the two approaches is a major strength of this work, but additional experiments would be useful to separate qE and other NPQ components from other non-radiative energy-loss processes.

5. Meaning of thermal resilience. The cooler *cia2cil* phenotype is biologically interesting, but reduced temperature can arise from lower pigment absorption and reduced metabolic/photosynthetic activity as well as from adaptive cooling. Long-term growth, fitness and reproduction under realistic combinations of high light, high temperature and drought would help determine whether the phenotype represents adaptive thermal resilience. Please comment on this.

6. Generalisation beyond *Arabidopsis*. The genetic conclusions are based primarily on *Arabidopsis thaliana*. The cross-species thermal measurements broaden the physiological perspective, but the transferability of CIA2/CIL mechanisms to crop or woody species remains an open question. Can the the Candidate provide some insight into possible application of the results to agriculturally relevant species?

7. Ecological and climate-change relevance. The thesis provides a useful mechanistic framework for considering plant thermal regulation under environmental stress. Nevertheless, controlled laboratory experiments and short-term treatments cannot fully reproduce field conditions. Longer-term, combined-stress experiments under field-like environments would be an important next step. Has any work been done in this direction?

Summary

This thesis presents a coherent investigation of the relationship between nuclear regulation of chloroplast biogenesis, photosynthetic development and foliar temperature. Its major contribution is the integration of molecular genetics with chloroplast structural analysis, photosynthetic physiology, thermal imaging and calorimetry.

The work demonstrates that CIA2 and CIL are required for normal chloroplast maturation and the timely establishment of photosynthetic competence. It further shows that photosynthetic processes, NPQ and transpiration jointly influence leaf temperature and that disruption of CIA2/CIL signalling can produce a cooler but physiologically compromised phenotype. The combination of these findings supports a systems-level view in which chloroplast development influences both energy use and heat management at the whole-leaf level.

Conclusions

Overall, the thesis represents a substantial and original contribution to plant molecular physiology, chloroplast biology and stress physiology. Its principal strengths are the clearly defined biological question, application of multidisciplinary yet complementary experimental approaches to verify the research hypothesis, the use of appropriate mutant and complementation lines, and the attempt to connect molecular regulation with measurable whole-organ thermal phenotypes.

The most important conceptual outcome is the proposed connection between CIA2/CIL-dependent chloroplast development, photosynthetic energy partitioning and foliar heat balance. The thesis also makes a valuable methodological contribution by combining fluorescence, thermography and photo-calorimetry to examine heat dissipation.

The main issues for further work concern the precise mechnistic interplay between chloroplast redox state and stomatal regulation, the specificity of pharmacological manipulation of NPQ, the environmental dependence of calorimetric estimates, and the adaptive significance of the cooler *cia2cil* phenotype. These points do not undermine the central findings; rather, they define interesting directions for future research.

In my assessment, the thesis is scientifically ambitious, methodologically strong and conceptually coherent. It provides meaningful new evidence on the role of CIA2 and CIL transcriptional components in chloroplast development, and establishes a valuable framework for understanding how photosynthetic energy management contributes to plant thermal regulation.

Overall assessment: In my opinion, the doctoral dissertation submitted by Ms Roshanak Zarrin Ghalami, MSc, addresses interesting, timely and important scientific problems and makes a significant contribution to the advancement of international science. It therefore meets the requirements applicable to doctoral proceedings and fulfils the conditions set out in the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended), and Resolution No. 89-2022/2023 of the Senate of the Warsaw University of Life Sciences (SGGW) of 26 June 2023 on the adoption of the Regulations governing proceedings for the award of doctoral degrees at the Warsaw University of Life Sciences.

Taking into account the considerable amount of work invested in carrying out this doctoral project, its strongly mechanistic character, as well as the significance, interdisciplinarity and innovative nature of the experimental and theoretical approaches employed to obtain such valuable research results, I would also like to recommend that this doctoral dissertation be awarded distinction.

Truly yours,

Joanna Kargul; Uniwersytet Warszawski

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