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Article Addendum - Plant Signaling and Behavior

Title: Adjustments of lipid pathways in plant adaptation to temperature stress

Authors: Qiang Li[1], Wenyun Shen[2], Qian Zheng[3], D. Brian Fowler[1] and Jitao Zou[2,*]

[1] Department of Plant Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5A8

[2] National Research Council Canada, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada S7N 0W9

[3] Department of Food and Bioproduct Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5A8

[*]To whom correspondence should be sent. Email: Jitao.Zou@nrc-cnrc.gc.ca

Corresponding author: Jitao Zou, National Research Council Canada, 110 Gymnasium Place, Saskatoon, Saskatchewan S7N 0W9. Phone 306-975-5583; Fax 306-975-4833; Email: Jitao.Zou@nrc-cnrc.gc.ca

Abstract:

Modulation of membrane lipid composition under varying environmental conditions is an important part of plant stress adaptation. Most notably, proportional changes of lipid composition in response to temperature changes are a major cellular response to requirements of membrane fluidity adjustment. In higher plants, synthesis of glycerolipids is accomplished by two major pathways, the prokaryotic and eukaryotic pathway, located in the chloroplast and the endoplasmic reticulum (ER), respectively. Recently, we systematically investigated the re-adjustments of glycerolipid pathways under temperature stress at the metabolite and transcript levels using three plant species with distinct lipid profiles. The relative contributions of two pathways and lipid channeling from the ER and chloroplast were both observed in plants under temperature stress. Potential factors controlling the lipid flux were identified through transcriptome analysis.

Keywords: fatty acid metabolism; glycerolipid pathways; temperature adaptation; RNA sequencing; metabolic regulation

Abbreviations: PG, phosphatidylglycerol; MGDG, monoglactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; C16, chain with 16 carbon; C18, chain with 18 carbon; DAG, diacylglycerol; TAG, triacylglycerol; ER, endoplasmic reticulum.

Addendum to: Li Q, Zheng Q, Shen W, Cram D, Fowler DB, Wei Y, Zou J. Understanding the biochemical basis of temperature-induced lipid pathway adjustments in plants. Plant Cell 2015; 27:86-103.

A key aspect of plant adaptation to temperature stress concerns modulation of membrane fluidity.[1-3] Membrane fluidity is affected by two key aspects of lipid composition: (1) the degree of unsaturation in the fatty acyl groups of glycerolipid molecules and (2) the relative proportions of various lipid classes in the lipid bilayer.[2-5] We recently conducted a comprehensive survey on glycerolipid metabolite as well as enzyme gene transcript changes under temperature stress in three plant species, *Arabidopsis thaliana* (Arabidopsis), *Atriplex lentiformis* (saltbush) and *Triticum aestivum* L. (wheat).[6] Our results show that glycerolipid pathway adjustment between the chloroplast and cytosolic compartments are fundamental to the modulation of membrane lipid attributes pertinent to temperature stress adaptation.

In plant cells, the cytosolic and chloroplast compartment each possesses its own set of glycerolipid assembly pathway and generates glycerolipid species of defined molecular characteristics.[7-9] The lipid pathway located in the chloroplast, which traces its origin to symbiogenesis, is called the prokaryotic pathway. The cytosolic endoplasmic reticulum (ER) lipid pathway, on the other hand, is known as the eukaryotic pathway. The two pathways are separated by barriers of the chloroplast envelope, but they are by no means insulated from each other. In fact, a significant portion of the glycerolipid moieties required for the biogenesis of photosynthetic membrane systems in the chloroplast is supplied by the eukaryotic pathway. The relative contributions of the two pathways, however, are very different among plant species.[10,11] In species like Arabidopsis, about half of the glycerolipid moieties in chloroplast come from the eukaryotic pathway.[8] In species, such as wheat, construction of photosynthesis apparatus relies

almost entirely on the eukaryotic pathway, while the contribution of prokaryotic pathway is limited to the production of phosphatidylglycerol (PG) only.[11,12] Despite the divergent pattern of relevant inputs from the two pathways, at the core is a basic requirement of glycerolipid channeling from the ER to chloroplasts in all plant species.

Changes in the proportion of glycerolipid classes under temperature stress have been previously reported in different plant species. The most common change is the adjustment between monoglactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), and it has been proposed that this metabolic change aids in survival at sub-optimal temperatures.[13] A greater proportion of DGDG has been observed at high temperatures in Arabidopsis, while low temperature induced the synthesis of MGDG in *Brassica napus*.[13,14] In addition to the alterations in the proportion of glycerolipid species, changes were also observed in the same lipid species with different fatty acid combinations. In *Brassica napus,* the major molecular species of MGDG is a mixture of *sn-1*18:3/*sn-2* 16:3 and *sn-1* 18:3/*sn-2* 18:3 molecule species.[8,11] An increase in MGDG (18:3/16:3) relative to MGDG (18:3/18:3) molecule species were observed in *Brassica napus* leaves grown at low temperature.[14] In contrast, a decrease in MGDG (18:3/16:3) relative to MGDG (18:3/18:3) was observed in Arabidopsis grown at high temperatures.[13]

Rebalancing of the two glycerolipid pathways influences both the distribution of glycerolipid classes of different head groups and the degree of fatty acid desaturation in glycerolipid molecules. Due to substrate specificity of the plastidic lysophosphatidic acyltransferase, the prokaryotic pathway produces glycerolipids with C16 fatty acyl moiety at the *sn-2* position of the glycerol backbone, and hence a molecular configuration of C34 (C18/ C16, *sn-1/ sn-2*).[8,11] The *sn-2* C16 fatty acids in the MGDG originating from the prokaryotic pathway are subsequently desaturated to form C16:3 fatty acids, leading to the formation of the highly unsaturated C34:6 (18:3/16:3) MGDG. The eukaryotic pathway generates glycerolipids with only C18 fatty acid at the *sn-2*, and produces C36 (C18/C18) and C34 (C16:0/C18). Since the *sn-1* 16:0 does not undergo any further desaturation, the eukaryotic C34:3 (16:0/18:3) has a level of desaturation much lower than that of the prokaryotic C34:6 (18:3/16:3). Through comprehensive lipid profiling and lipidomics analysis, we have shown that high temperatures repress the input of the prokaryotic pathway and promote the channeling of eukaryotic lipids to the chloroplast, thereby reducing potential of fatty acid desaturation in the photosynthesis apparatus. Significantly, we also observed that there was a differential channeling of eukaryotic

glycerolipid species from the ER to the chloroplast: C34 diacylglycerol (DAG) moieties of 16:0/C18 (*sn-1/ sn-2*) from the ER were preferentially transported to the chloroplast over that of C36 (C18/C18) under high temperature. These two layers of metabolic adjustments collectively reduce the overall degree of fatty acid desaturation in chloroplast lipids, particularly that of MGDG, at higher temperature. In wheat, where chloroplast lipid synthesis is dependent completely on imports from the ER, a differential channeling of C34 (C16/C18) DAG versus C36 (C18/ C18) DAG was also observed. Under low temperature, there was a reduction of C34 DAG with a corresponding increase of C36 DAG. This metabolic alteration increased the overall potential of glycerolipid unsaturation in the chloroplast, i.e. increased level of C36 (18:3/18:3). The California desert plant *A. lentiformis* presents an exceptional case, which shuts off its prokaryotic pathways at high temperature, and falls back on the eukaryotic pathway completely for chloroplast lipid synthesis.[15] This metabolic rebalancing allows the ER to deliver more C34 DAG (16:0/C18), thereby reducing the degree of lipid desaturation under extreme heat. These three species demonstrate the essential role glycerolipid pathway adjustment during adaptation to temperature stress.

Transcript analysis of our study and previous reports showed that, consistent with changes observed at the metabolite level, glycerolipid pathway coordination was reflected at the level of gene transcripts.[16,17] The prokaryotic glycerol-3-phosphate acyltransferase *ACT1* [18,19] and the chloroplast desaturase *FATTY ACID DESATURASE 5* (*FAD5*)[20,21] are key components influencing the prokaryotic glycerolipid pathway, while the *FATTY ACID DESATURASE 2* (*FAD2*)[22-24] in the ER was tightly associated with activities of the eukaryotic pathway. In addition to polar lipid biosynthesis pathways, we also detected a large number of differentially expressed genes involved in other branches of lipid metabolism (**Figure 1**).[6, 25] Triacylglycerols (TAGs) in Arabidopsis leaf tissues has been implicated in short-term glycerolipid intermediate provision during membrane lipid remodeling.[26,27] Accumulation of TAGs in plant leaves has been reported in Arabidopsis under freezing temperature.[28] In wheat, about 50 genes related to TAG synthesis were induced at 4 ^{o}C. This was contrasted by only a few genes affected under heat at 43 ^{o}C in *A. lentiformis*. We also detected up-regulation of a large number of genes involved in fatty acid elongation and wax biosynthesis at 4 ^{o}C in wheat. In both *A.lentiformis* and wheat, significant numbers of phospholipid signaling genes were perturbed, suggesting active lipid signaling and lipid transfer events in this process.

Our work draws attention to the importance of glycerolipid pathway coordination during adaptation to temperature stress. Differential channeling of DAG moieties with various combinations of fatty acid moieties from the ER to chloroplast presents another layer of regulatory fine tuning for membrane unsaturation in chloroplast. A challenging question is why plants possess so many desaturase genes but still depend on glycerolipid pathway adjustment to modulate membrane desaturation. The simplest explanation would be that different intracellular compartments as well as developmental and tissues specific regulations demand the participation of multiple members of desaturase. Members of the desaturase family generally have defined glycerolipid substrate and/or fatty acid chain length specificity [29, 30]. Hence, their roles in mediating the desaturation of non-glycerolipids components of membrane systems during stress adaptation are also important. [30, 31] We suggest that specification of desaturases and pathway integration are two sides of one regulatory regime; together it allows the membrane system responding to temperature changes, but not in a fashion of a one way extreme, because in nature temperature fluctuates constantly. Adjustment of the two pathways provide a framework for desaturases to impart membrane compositional changes, but at the same time, stabilizes the lipid metabolic network against drastic changes to ensure biological robustness. Questions remain as to how lipids are selectively transported between chloroplast and the ER. In addition, the roles of other lipid species such as triacylglycerol, wax, oxylipin are also worthy topics for further investigation.

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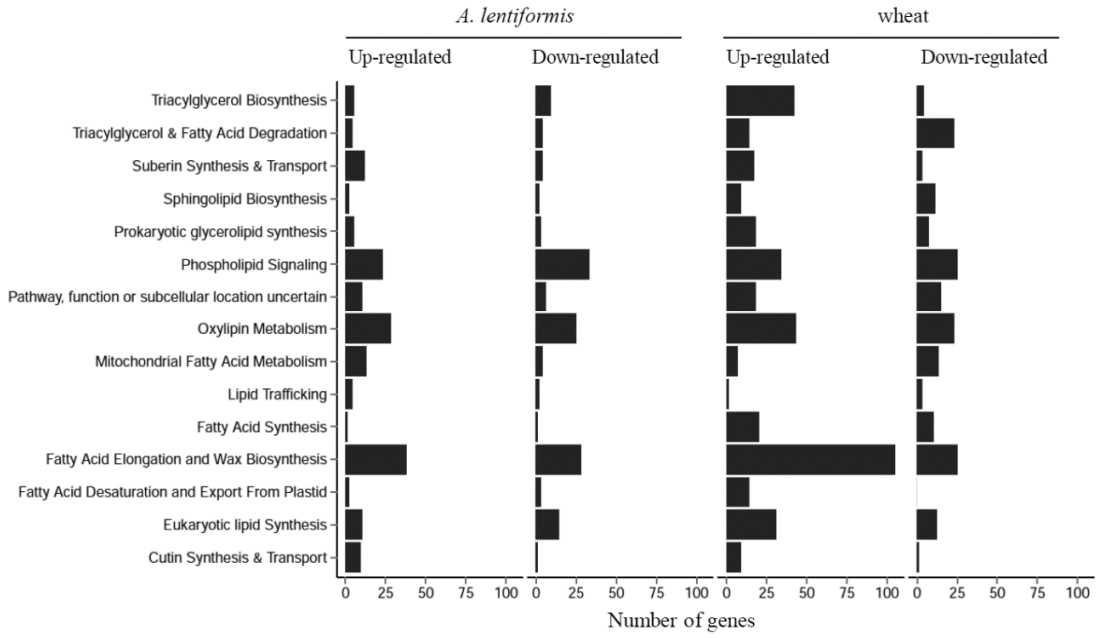
Figure Legend

Figure 1 Number of lipid metabolism genes with significant changes in plants grown at low or high temperatures. *A.lentiformis* plants were grown at 43 ºC (high temperature) and 23 ºC (control) while wheat plants were grown at 4 ºC (low temperature) and 23 ºC (control). RNA sequencing was performed for *A.lentiformis* and wheat at high or low temperature respectively. Differentially expression genes (fold change >2 or <-2, p value <0.05) in *A.lentiformis* (43 ºC vs 23 ºC) and wheat (4 ºC vs 23 ºC) were identified.[6] Genes involved in lipid metabolism were compiled from the Arabidopsis Lipid Gene Database (http://aralip.plantbiology.msu.edu/) [25] and categorized based on different pathways.

Figure 1


A. lentiformis
wheat
Up-regulated
Down-regulated
Up-regulated
Down-regulated
Triacylglycerol Biosynthesis
Triacylglycerol & Fatty Acid Degradation
Suberin Synthesis & Transport
Sphingolipid Biosynthesis
Prokaryotic glycerolipid synthesis
Phospholipid Signaling
Pathway, function or subcellular location uncertain
Oxylipin Metabolism
Mitochondrial Fatty Acid Metabolism
Lipid Trafficking
Fatty Acid Synthesis
Fatty Acid Elongation and Wax Biosynthesis
Fatty Acid Desaturation and Export From Plastid
Eukaryotic lipid Synthesis
Cutin Synthesis & Transport
0 25 50 75 100
Number of genes

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