


SHORT COMMUNICATION



Membrane lipids are involved in plant response to oxygen deprivation

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ABSTRACT

Membrane lipids change drastically in plants when they suffered from hypoxia (oxygen deficiency) stress. Overall, hypoxia stress lowers the contents of total lipids, inhibits lipid biosynthesis, and stimulates lipid degradation, leading to the accumulation of free fatty acids. Lipid alterations include changes in the contents of lipid classes, the extent of saturation, and the length of acyl chains. But the detail and systematic studies about lipid changes, as well as the function mechanism in hypoxia stress are poorly understood. Here, the major unanswered questions and suggestions on the study of the function of lipid in hypoxia stress were provided.

ARTICLE HISTORY

Received 22 February 2020
Revised 28 April 2020
Accepted 29 April 2020

KEYWORDS

hypoxia stress; lipid metabolism

The galactolipids, phospholipids, and sphingolipids compositions are involved in hypoxia stress

Membrane lipids change drastically in plants when they suffered from hypoxia stress and the lipid contents variation under hypoxia stress is our first concern.¹ We focus on the changes in galactolipids, phospholipids, and sphingolipids compositions with significant abundance and importance. The photosynthetic membranes of higher plant chloroplast consist of four main classes of glycerolipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), the phospholipid phosphatidylglycerol (PG), and the sulfolipid sulfoquinovosyldiacylglycerol (SQDG). Phospholipids and galactolipids of photosynthetic membranes in plastids are synthesized by the prokaryotic pathway, while lipids of extraplastidic membranes are produced in the endoplasmic reticulum by the eukaryotic pathway.²

In *Arabidopsis*, a significant decrease in the total amount of DGDG and MGDG was reported under hypoxia stress,^{3,4} and it was also supported by our previous findings in wheat.⁵ MGDG and DGDG are closely related to thylakoid membrane structure and photosynthetic properties of plants. Besides, the changed abundance of galactolipids may be responsible for altered photosynthetic membranes.⁶ Klecker et al. reported that a 24 h hypoxia treatment induced the transcript levels of two MGDG synthases genes *MGD2* and *MGD3*, as well as the major DGDG synthase *DGD1* gene in *Arabidopsis*. However, the total galactolipid contents were not severely affected and the phenotype of *mgd2*, *mgd3*, and *mgd2/mgd3* mutants was similar to wild type under hypoxia stress.⁷ In contrast, DGDG accumulated in *Amaranthus paniculatus* upon mild hypoxia (4% O₂) for an extended period of 21 d.⁸

Except for the absolute contents, the molecular species of DGDG and MGDG as well as DGDG: MGDG ratio are also involved in hypoxia stress. For instance, the content of 34:6

(18:3, 16:3) MGDG slightly increased during submergence, accompanied by a decrease in 36:6 (18:3, 18:3) MGDG, while DGDG showed an inverse trend. Two pathways are employed for galactolipid synthesis in *Arabidopsis*: 36:6 molecular species are ER derived and 34:6/34:3 molecular species are chloroplast derived.⁷ These changes in galactolipid composition suggest a redistribution of lipid species originating from the ER and the chloroplast during hypoxia stress. In addition, the increase of DGDG: MGDG ratio may alter membrane permeability and fluidity in abiotic stress like drought and copper tolerance.^{6,9} The DGDG: MGDG ratio also increases under hypoxia stress.^{3,4}

The content and composition of sulfolipid SQDG did not change during submergence, though light-exposed *Arabidopsis* seedlings showed an increased expression of *SQD2* gene involved in sulfolipid metabolism during hypoxia treatment.⁷

Membrane phospholipids also underwent very rapid and severe deterioration during hypoxia stress. Researches in *Arabidopsis thaliana* showed that a short-term hypoxic treatment induced a significant increase in the total amounts of phosphatidylserine (PS) and phosphatidic acid (PA), together with a significant decrease in the total amounts of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and other species of phospholipids. The accumulation of PA species was correlated with the decline in other species (PC, PE, etc.), indicating the enhanced role of phospholipase D (PLD), which hydrolyzes phospholipids into PA and a head group. This mechanism is similar to desiccation, freezing, and salt stress.^{10–14} Moreover, knockout mutants of PLD δ displayed more sensitive to hypoxic stress than the wild type (unpublished data). Studies in *Arabidopsis* have revealed that only small differences existed in lipid contents between the control and submerged plants for 1 d, but dramatic degradation occurred after 3 d of submergence which is in line with the phenotype.⁴

Furthermore, studies in *Arabidopsis* have reported a significant increase in the polyunsaturated molecular species of PC, PE, and phosphatidylinositol (PI) and a decline in their saturated and monounsaturated molecular species, suggesting that lipid desaturation occurs when plants are exposed to hypoxia.^{7,15} The higher proportions of unsaturated fatty acid compositions of the wax esters, triglycerides, and steryl esters were also present in submerged mangrove plants.¹⁶ However, Wang et al. reported submergence did not affect membrane fluidity based on the evidence obtained from the double bond index.⁴ Whether the desaturation of fatty acid compositions of lipids was involved in hypoxia stress remains to be answered.

Lyso-phospholipids are derived from phospholipase A-mediated hydrolysis at the sn-1 or sn-2 position of phospholipids.¹⁷ The lysophospholipids exhibited various patterns of changes induced by submergence and it is different from the findings obtained from freezing and dehydration stress.⁴ Basically, it is difficult to quantify the contents of lysophospholipids accurately due to their low concentrations.

We summarized the lipid profiling (galactolipids and phospholipids) results in researches published recently including our previous data in Supplementary Table 1. Based on the common trend, the possible changes in these lipids during submergence treatment could be predicted.

As for the sphingolipid, the modification of the very long-chain unsaturated ceramides is a protective strategy for hypoxic tolerance through the modulation of CTR1-mediated ethylene signaling, which was reported for the first time in *Arabidopsis*.³ Ceramide and hydroxyceramide levels increased significantly under hypoxia stress. The *Arabidopsis* ceramide synthase *loh* mutants exhibit enhanced sensitivity to dark submergence due to their low levels of ceramide species containing VLCFAs (very long-chain fatty acids, 22:1, 24:1, and 26:1), and *myb30* knockout mutants (MYB30 is responsible for the regulation of VLCFA biosynthesis) show hypersensitivity to submergence with light.³

Submergence triggered a significant decrease of wax and cutin compounds in rosettes. Deletion of *Arabidopsis* long-chain acyl-CoA synthetases 2 (LACS2) resulted in reduced cuticular wax and thinner cutin layer and attenuated plant resistance to submergence stress.¹⁸

Possible mechanism of changes in lipid composition under hypoxia stress

Lipids store energy, form cell membranes, and modulate plants' responses to abiotic and biotic stress. Lipids and lipid-derived metabolites interact with proteins to regulate enzyme activity and/or transcription. These changes have the potential to mediate and/or modulate plants' responses to stress.

The reduced rate of lipid turnover under anoxia is presumably due to the inhibition or low activities of lipases. The falling ATP concentration under anoxia has been shown to act as a threshold regulatory switch for membrane integrity: when ATP concentration decreases below a certain value, the integrity of membrane lipids is no longer preserved and they are hydrolyzed to free fatty acid. The liberation of free fatty acids is an indicator of severe membrane damage and manifests as cell death.¹⁹

Lipids possess two major roles in plants' response to stress. Firstly, they act as signaling mediators including PA, PIs, sphingolipids, lysophospholipids, oxylipins, N-acyl ethanolamines, free fatty acids, and others.^{20–24} Signaling lipids often occur at very low levels and display a quick turnover upon stimulation. Among them, PA is the most versatile one, and more than 20 PA binding proteins were involved in diverse cellular processes like lipid metabolism/transport, phytohormones signaling.²⁵ PA contents were significantly induced under hypoxia stress similar to other stresses.^{3,4,15}

Secondly, membrane lipids remodeling maintains lipid dynamics and membrane protein functionality in response to abiotic stresses.^{26–28} Types of lipid alteration include changes in the contents of lipid classes as well as the degree of unsaturation (number of double bonds) in the fatty acyl groups. These changes directly affect membrane fluidity and integrity.²⁹ Different lipid classes have varied potential to form hexagonal II (HII) phase or maintain bilayer structure. Lipids such as MGDG and PE tend to form HII phase or other non-bilayer phases, whereas DGDG, SQDG, PC, and PG form bilayers.^{30–33} Plants try to decrease the degree of unsaturation at high temperatures while the trend was opposite under freezing stress to maintain optimal fluidity and integrity of membranes.^{29,34} Whether the same or similar mechanism was involved in hypoxia stress response like well-studied freezing and drought stress remains to be solved.

Conclusions and perspectives

Although we are sure that lipid changes were involved in hypoxic tolerance in plants, the exact details of lipid remodeling, regulatory network, and mechanisms remain to be further investigated. We have summarized the unsolved problems as follows:

The details of the responses of membrane lipid and the changes in membrane structure to hypoxia stress are still unknown. An integrated lipidomic and transcriptomic analysis can be conducted to investigate the regulation of lipids metabolism at both biochemical and molecular biological levels in plants to obtain better understandings of the roles played by lipid remodeling in hypoxia response.

The exact underlying mechanisms of membrane lipid remodeling in mediating hypoxia response also need better answers. Plants' responses to different abiotic stresses are often very specific, but signal transduction pathways could partially or completely overlap. Lipid signaling under drought, salt, cold, nitrogen, phosphorus deficiency, and heavy metal stress has been extensively studied. The core difference at the cellular level between submergence and other stresses is water potential. The different water potentials influence the hydrophobic force of lipids, making them form laminar membranes. Whether the signaling roles of lipids or the intermediates in plant hypoxia stress response were the same with extensively studied stress like freezing and drought stress remains to be answered.

Collectively answering these questions will allow us to identify the key lipids and pathways responsible for resilience, enabling the improvement of crop tolerance to hypoxia stress. We also offer some suggestions for further study based on current knowledge about the lipid remodeling mechanism under low oxygen stress.

It is obvious that the contents, species, and degree of saturation of different lipids in plants varied during hypoxia treatment. When using different treatment methods (waterlogging, submergence, and gas control to mimic the hypoxic conditions),³⁵ harsh extent, and short or long durations, different phenomena and responses of membrane lipids were observed. The development stage of plants, the growth and treatment conditions (light and temperature), etc. may also lead to different responses in plants. All these factors should be taken into consideration.

In addition, the response pattern may vary among different plant species, and it even could be different in sensitive and tolerant plants of the same species. The extent of anoxia-induced lipid loss is lower in resistant genotypes than in non-resistant genotypes. For plants with a library of mutants like rice and *Arabidopsis*, we should make full use of mutants in lipid pathway, and for those species without ample mutants, it is preferred to use varieties with distinct tolerance of the similar genetic background (at least two pairs). In addition, technologies such as genome editing (e.g. CRISPR/Cas9) can be utilized to modify the gene of interest in a precise manner.^{36,37}

The oxidative modifications of lipids are the main cause of membrane destabilization and injury, indicated by increased electrolyte leakage under a range of stimuli including hypoxia stress.³⁸ Anoxic stress may induce qualitative changes in membrane lipids, as indicated by lipid peroxidation after the restoration of aerobic conditions. A burst of reactive oxygen species may be formed immediately after oxygen reenters the tissues. Besides, the degradation of lipids and free fatty acids facilitated by lipid peroxidation is activated mainly after reoxygenation.³⁸ Most recent studies have focused on the hypoxia stage, while the reoxygenation stage has been largely ignored, which deserves more attention.

Reoxygenation in *Arabidopsis* results in increased transcript levels of jasmonic acid (JA) biosynthesis genes and the rapid accumulation of jasmonates, and JA signaling interacts with the antioxidant pathway to regulate reoxygenation responses.³⁹ The free fatty acids produced by phospholipase A are the precursors for the synthesis of oxylipins such as JA and 12-oxo-phytodienoic acid. And there is also a strong link between galactolipid generation and JA accumulation.⁴⁰ The crosstalk between lipid metabolism and phytohormones (esp. lipid-derived ones) may be a promising direction.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the National Science Foundation [31901438].

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