

The effect of low temperature on metabolism of membrane lipids in plants and associated gene expression

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Abstract

Lipid metabolism plays an important role in the mechanism of frost or cold-tolerance in plants. Plant membrane lipids have the tendency to change from gel to liquid-crystalline phase in response to low temperature stress. This process is due to the increased level of lipid desaturation. The responsible components of this process are, among others, the fatty acid desaturases. Controlling the activity of these enzymes affects the amount of polyunsaturated fatty acids on the glycerol backbone and eventually controls the plants sensitivity to low temperature stress. These metabolic processes trigger a series of changes at the transcriptional level, causing differential expression in genes. Numerous approaches towards this process from chemical to the advanced mass spectrometry were taken during the past decades and several of them will be discussed in this minireview. Metabolomics and transcriptomics seem to be the keys towards describing these complex mechanisms and providing the necessary understanding of lipid metabolic response to low temperature stress.

Keywords: Lipid metabolism; desaturase enzymes; desaturation; gene expression.

Abbreviations: ABA_ abscisic acid; DAG_ diacylglycerol; DBI_ double bond index; DGDG_ digalactosyldiacylglycerol; EL_ electrolyte leakage; ER_ endoplasmic reticulum; FS_ free sterols; GL_ glycolipids; MDA_ malonic dialdehyde; MGDG_ monogalactosyldiacylglycerol; PA_ phosphatidic acid; PC_ phosphatidylcholine; PE_ phosphatidylethanolamine; PI_ phosphatidylinositol; PL_ phospholipids; pr_ lipids/proteins ratios; ROS_ reactive oxygen species; VLCPUFA_ very long chain polyunsaturated acids

Introduction

When exposed to different stresses, plants have different ways of dealing with it to compensate for their lack of mobility. Cold is an abiotic stress that has been intensively studied in plants because of its negative effects on plant growth and development which is related to yield and productivity rates in cultivated crops. This is a limiting factor for growing plants from warmer areas in different climatic areas where the temperature is less suitable for cultivation.

The plants response to cold varies from species to species and even within the same plant family. However, regardless of plant species complexity, one major common mechanism used by plants to deal with cold stress is changes in membrane lipid composition to protect its membrane stability and integrity. It was reported that there is also a great accumulation of soluble sugars in response to cold stress (Deryabin et al. 2005) and even the major role

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that is played by light in this complex response mechanism (Zhu et al. 2007). These metabolic processes trigger a series of changes at the transcriptional level, determining differential expression in genes. It is believed that deciphering the mechanism of membrane lipids of plants exposed to low temperature will help in finding the answer to cold stress that could be a major breakthrough for the future of agriculture.

This mini review highlights the efforts of several groups in studying the membrane lipids changes during cold acclimatization and its effect on the gene expression in different plant species, such as Adzuki beans (*Vigna angularis* (Wild.) Ohwi & Ohashi), Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.), chickpea (*Cicer arietinum* L.), coffee bean (*Coffea* sp.), cucumber (*Cucumis sativus* L.), figleaf gourd (*Cucurbita ficifolia* Bouché), potato (*Solanum tuberosum* L.), rapeseed (*Brassica napus* L.), rice (*Oryza sativa* L.), soybean (*Glycine max* (L.) Merr.), and winter wheat (*Triticum aestivum* L.) (Table 1). The results and conclusions and the correlations among these variety of plants are discussed and compared in order to give a broader picture of the mechanism of membrane lipids metabolism. However, despite the fact that the approaches taken and the plant material were differed among the research groups in the end their results were, surprisingly, quite alike.

Changes in plants in response to low temperature stress

It is known to a great extent that one of membrane lipids response action when exposed to cold would be the increase in the unsaturation of fatty acids compared to fatty acids in plant under normal conditions (Tasseva et al. 2004; De Palma et al. 2008). However, this ratio differs substantially from plant to plant and even the same plant can act differently depending on the way of exposure to low temperature (i.e. acclimated (3-4°C) vs. non-acclimated plants (22-24°C)). Most of the research focused on the impact of cold on leaves and roots.

Rapeseed

Tasseva et al. (2004) have studied the lipid changes in response to cold in the endoplasmic reticulum (ER) of rapeseed. They observed an increase in the level of unsaturation when the plants were grown at 4°C. Choosing the ER for their analysis they were hoping

that would provide more insight on the lipid metabolism, knowing that ER is the site for lipid biosynthesis. They have studied the action of the desaturases and acyltransferases after cold stress. For the experiment they studied frost-tolerant and -sensitive cultivars, respectively. They used the double bond index (DBI) to measure the unsaturation levels of the lipids for analyzing their results. There were not major differences in lipid changes between the two cultivars with the exception of the increase (3%) in the very long chain polyunsaturated acids (VLCPUFA) in the sensitive plants at 4°C. Overall, there was an important increase in linoleic (18:2) and alpha-linolenic (18:3) acids in both types of studied plants when exposed to low temperature. However, what is notable is the time of accumulation of these acids in the tested plants. The tolerant plants showed a very fast accumulation in unsaturated acids, after only a few days compared to the sensitive ones, conferring a more rapid adaptation to cold. This could lead to the conclusion that the tolerant plants have a better responding mechanism to reduce cold stress membrane damages. To better understand this process, Tasseva et al. (2004) performed analysis at the transcriptional level, too. In this way they found that there were changes in the transcript level of certain enzymes involved in lipid metabolism. There was a substantial increase in stearoyl-ACP desaturase isogene level that was orthologous to *At1g43800* gene. All the other enzymes did not show major difference in transcript level when compared to results from the sensitive plants. They concluded that this desaturase played a major role in the cold-tolerance mechanism of membrane lipids.

Potato

The implication of the stearoyl-ACP desaturase has also been, recently, studied by De Palma et al. (2008) and the implication of this desaturase gene in changing the composition of the membrane lipids in potato plants. Their work suggests that the expression of the stearoyl-ACP desaturase (*w-9*) gene in plants will increase the cold tolerance in plants due to the increased desaturation of the fatty acids and thus a better membrane control of damage at the membrane level. They have compared transgenic plants carrying *w-9* gene and non-transgenic plants that were either cold-acclimated or non-acclimated. They found that plants with increased cold tolerance showed a substantial increase in linoleic acid (18:2) compared to the more sensitive plants that presented an

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increased in alpha-linolenic acid (18:3). They suggested that the major component of this mechanism was the level of desaturases in the plants. However, upon fatty acid analysis of the plants they observed that the extrachloroplastic membranes of the transgenic plants contained, among the normal fatty acids, a cis-vaccenic acid (18:1 Δ 11) in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) which is unusual in plants (3%). They believe that the presence of this peculiar fatty acid is due to the excess of the stearyl-ACP desaturase as previously stated by Tasseva et al. (2004). Overall, the membrane lipids upon stress have changed their composition showing increase (by 5 to 10%) in unsaturated fatty acids in potato.

Potato plants were also used as a material to study cold stress and changes in membrane lipids. Deryabin et al. (2005) used transgenic plants that contained a yeast-derived invertase gene (*B33-inv* plants) to study peroxidation of membrane lipids exposed to low temperatures. Invertases are enzymes that are involved in sugar metabolism that catalyses the hydrolysis of sucrose into glucose and fructose. They focused on the importance of soluble sugar in lipid peroxidation due to low temperature exposure. Lipid peroxidation has been measured as the accumulation of its secondary product malonic dialdehyde (MDA) in potato leaves. Analyzing the plants after chilling (3°C) they have found that there is a slight increase in MDA in both *B33-inv* plants (tolerant) and non-transgenic (sensitive) plants with a higher content (17%) in the sensitive plant. However, after decreasing the temperature (-1°C) they observed an even higher increase (62%) for the sensitive compared to tolerant plants. They have concluded that this low increase in MDA for the transgenic plants is due to the invertase gene and that it plays an important role in tolerance to low temperatures. Their work did not provide any information on lipids classes' changes in detail but it gives important information on other factors, such as sugar interaction, involved in membrane lipid behavior when exposed to cold stress.

Winter Wheat

Bohn et al. (2007) studied the effect of cold on the plasma membrane lipids of winter wheat. It is known that the recovery of the plant after exposure to low temperature during winter is critical for its productivity. Their work focused on two different methods to induce cold, either by exposure to low

temperature or by treatment with the hormone abscisic acid (ABA) on both cold- and non acclimated plants. The plant material chosen for testing had different levels of cold tolerance. Three major lipid groups were taken into consideration, such as free sterols (FS), glycolipids (GL) and phospholipids (PL) when applying cold treatment. Changes in these groups were observed during the experiment. In their study, they measured changes in lipids/proteins (pr) ratios between plants (stressed vs. non-stressed, cold- vs. non-acclimated and cold induced vs. ABA induced stress). The results are quite different and showed that the stress response mechanism is very complex. Moreover, they have found that the way of inducing the stress plays an important role in the plant response.

Without taking into consideration their differences in percentage of lipids between cold-tolerant and -sensitive plants, the only major difference in lipid/protein ratios was that of GL/pr for the tolerant plants. However, plants from etiolated seedlings stressed by ABA presented different protein ratios for almost all lipid classes, FS, PL, GL, TL, while only classes for GL were identified by tolerant plants derived from green seedlings. These are very important findings for biotechnology, knowing that hormone manipulation is considered one of the many options in genetic engineering. Regardless of the stress-inducing method, there have been notable changes in membrane lipids upon stress. The amount of free sterols increased in all plants, as well as the phospholipids, with the exception of the susceptible plants. Glycolipid amount showed a decrease for all tested plants. The amount of alpha-linolenic acid (18:3) doubled for the resistant plant in PC and PE. However, the amount of linoleic acid (18:2) increased in PL for the same plants. This confirms the findings of De Palma et al. (2008) on potato tolerant plants. Analogous experiments with similar results involving the role of ABA on the desaturation of fatty acids were performed by Bakht et al. (2006) on chickpea.

Rice

Rice is another species that has been studied for low temperature stress and its influence on membrane lipids. Zhu et al. (2007) have studied the unsaturated levels of fatty acids in lipids of the chloroplastic thylakoid membrane. After studying three rice cultivars with different levels of resistance they observed positive correlations between the unsaturated fatty acids (18:1, 18:2 and 18:3) and the

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Table 1. The diversity of cold tolerance on of lipid membrane metabolism in different plant species

Plant species	Scientific names	Plant families	Genes reported	Plant part or cell organelle targeted	References
Rapeseed	<i>Brassica napus</i>	Brassicaceae (D)	<i>w-9</i>	Endoplasmic reticulum	Tasseva et al. (2004)
Potato	<i>Solanum tuberosum</i>	Solanaceae (D)	<i>w-9</i> <i>B33-inv</i>	Stems Leaves	De Palma et al. (2008) Deryabin et al. (2005)
Winter wheat	<i>Triticum aestivum</i>	Poaceae (M)	C-repeat binding factors, dehydration responsive elements, abscisic acid responsive elements	Seedlings	Bohn et al. (2007)
Rice	<i>Oryza sativa</i>	Poaceae (M)	-	Chloroplastic thylakoid membrane	Zhu et al. (2007)
Adzuki beans	<i>Vigna angularis</i>	Fabaceae (D)	-	Root cell suspension culture	Kojima et al. (1998)
Coffee	<i>Coffea</i> sp.	Rubiaceae (D)	-	Whole plants	Campos et al. (2003)
Soybeans	<i>Glycine max</i>	Fabaceae (D)	-	Thylakoid from leaves	Tambussi et al. (2004)
Cucumber	<i>Cucumis sativus</i>	Cucurbitaceae (D)	-	Roots	Lee et al. (2005)
Figleaf gourd	<i>Cucurbita ficifolia</i>	Cucurbitaceae (D)	-	Roots	Lee et al. (2005)
Arabidopsis	<i>Arabidopsis thaliana</i>	Brassicaceae (D)	<i>PLDa1</i>	Plant tissues (specific part not reported)	Wang et al. (2006)

(M) = Monocotyledonous; (D) = Dicotyledonous

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electron transport activities, D1 protein content and F_v/F_m (PSII variable to maximum fluorescence). Conversely these correlations were negative for the saturated acids (16:0, 18:0). They concluded that the increase in unsaturation during low temperature definitely had a positive impact on repairing PSII from damage from chilling (11°C) with an important role in preserving the integrity of the membranes.

Adzuki Beans

Different research groups have studied different bean species for cold tolerance with regards to membrane lipids changes. Kojima et al. (1998) studied the effect of low temperature on Adzuki beans membrane lipids by tissue culture for a better uniformity and lower external factors other than the stress induced. They observed, among other modifications, the changes in lipid species of PC, PE and PI of phospholipids; of MGDG and DGDG of glycolipids; and of free sterols. Acclimatization at 3°C of bean cell culture did not have the same growth rate as the ones grown at room temperature (24°C). Moreover, lipid composition was lacking in certain classes such as PA and DAG. Of the free sterols, sitosterols and stigmaterols present in culture at 3°C were the most distinct compared with samples of cells cultured at 24°C. This had been previously reported by Guye et al. (1988) as directly related to low temperature tolerance. Moreover, Kojima et al. (1998) have noticed the increase in unsaturated acids in DAGs, especially in PC and PE and less in PI in the cell culture exposed at 3°C.

Coffee Beans

Campos et al. (2003) focused on the lipid degradation in coffee beans due to cold stress. They tested five different coffee genotypes with different tolerance to low temperature stress. To measure the peroxidation levels they used the classical approach of determining the amount of MDA, as secondary product of peroxidation. As expected the sensitive genotype showed the highest percentage of MDA. For analysis they have used four different temperature settings, the lowest being 3°C. The degree of unsaturation and the changes in lipid classes were targeted. In addition the DBI values were calculated for the studied genotypes. Two genotypes presented higher DBI values than the others, being the ones more tolerant to cold. They observed that as the temperature decreased the glycolipids ratio MGDG/DGDG decreased especially in the more tolerant plants suggesting that this, together with the increase in DGDG, might be

correlated with stability of the membranes. Conversely, the sensitive genotype showed a decrease in DGDG which can only enforce the above statement. As expected, increase in unsaturation was observed among all genotypes. However, the highest percent of alpha-linolenic acid (18:3) was observed in the more tolerant ones which is in contrast with recent findings in other species (Bohn et al. 2007, Deryabin et al. 2005).

Soybeans

Soybeans were also studied for oxidative stress during cold stress. Tambussi et al. (2004) studied the amount of reactive oxygen species (ROS) as an effect to membrane response to low temperature stress. It is known that the production of ROS is directly related to abiotic stresses. The increase in production of ROS is a well known mechanism in stress defense that will cause a cascade of oxidative processes that are detrimental to lipids, due to the increased peroxidation processes. Their approach was to calculate MDA levels in thylakoid from soybean leaves under cold and normal temperature. However, in contrast to other studies there was no increase in MDA after cold stress. This could also be due to the choice of the cultivar (tolerant), knowing that this increase was observed in cold-sensitive plants in other species (Bohn et al. 2007, Deryabin et al. 2005).

Cucumber and Figleaf Gourd

Lee et al. (2005) studied the lipid unsaturation and lipoxygenase activity after cold stress between a cold-tolerant (figleaf gourd) and a sensitive specie (cucumber). They have targeted the roots and not the leaves. Their results were very interesting with regards to the electrolyte leakage (EL) and DBI values, and the C18/C16 ratio. When comparing DBI values for several days they observed that the tolerant plant had a very uniformly ascending scale and a low and relatively constant EL. However, in the sensitive plants, the DBI values were quite variable with lowest value on the second day and the highest value on the third day of the stress. Moreover, the values of EL were constantly increasing after the second day. Based on these results they suggested that a gradual increase in DBI could be positively correlated with the tolerance to low temperature. Also the ratio of C18/C16 differed evidently between the two plants. They reported that the increase in C18:3/C16:0 was followed by a decrease in C18:0/C16:0 whereas the increase in C18:3/C16:0 was not correlated with the

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decrease in 18:0/C16:0 in the susceptible specie, the latter being constant.

Arabidopsis thaliana

Wang et al. (2006) have taken into consideration newer methods such as profiling gene expression upon cold stress on *Arabidopsis* and they have tried to take advantage of the completely known genome sequence of this plant. They compared known sequence profiles with the one of stressed plants, trying to find out up-regulated genes involved in low temperature stress that cause changes in membrane lipids. In this way they found out the involvement of some phospholipases, especially PLD which led to increases in the amount of PA during stress. However, of the 12 genes responsible for PLD in *Arabidopsis*, they identified that only *PLDa1* was responsible for PC hydrolysis and PA production. There was no association for the PLD genes with the PE or PG. This work is a great example of using available genome sequence information to find out new and reliable information about the enzymes implicated in this process.

Conclusions and future directions

Even though we have a broad knowledge on lipids, lipid species, and their metabolism and roles, there are still plenty of unknowns as how these mechanisms are triggered when exogenous factors interact with them. It is already clear that there is one common effect of low temperature stress on membrane lipids among most plant species that is the increase in unsaturation level of the fatty acids. Moreover, there could be a possibility of having a pattern in this increase of unsaturation with the more tolerant plants correlated with increased linoleic acid (18:2) and the susceptible ones correlated with alpha-linolenic (18:3). Also, there have been important findings of enzymes, such as stearoyl-ACP desaturase, *PLDa1* lipase, and even invertase, involved in the lipid changes mechanism, and their associated gene expression, that helped in verifying their involvement in this complex process. However, the science of metabolomics is using the metabolite profiling approach to identify more genes involved, among others, in lipid metabolism. It is clear that a combined approach between the modern techniques of biotechnology and a collective effort will lead towards a better understanding of this cold stress response mechanism.

Abiotic stresses, such as low temperature stress are among the most important research area lipid metabolomics. The limited amount of agricultural land and the constant increase in the world population is urging researchers to find a solution that is becoming more than just breaking the science barrier, but more a matter of survival for the future generations. It is very clear that understanding the lipid mechanism to respond to cold stress and designing plants tolerant to cold stress could be the key in, at least, partially solving the land-shortage problem by allowing plants to be cultivated more broadly in the inhospitable colder temperate regions of the world.

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