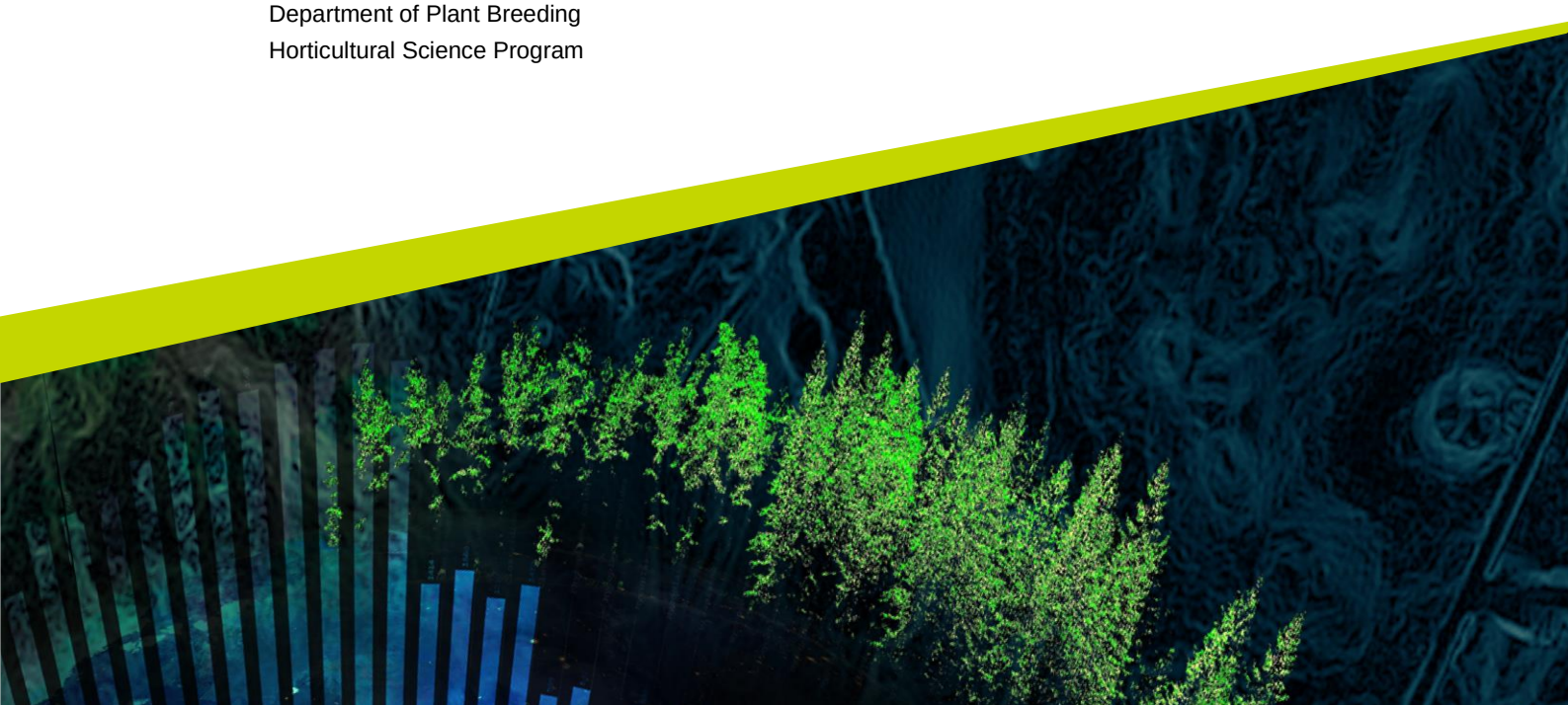
Finding the physiological function of the enzyme GPCAT.

– A gene expression study using Arabidopsis plants with silenced GPCAT gene

Ida Hallström

Independent project in Biology, Bachelor thesis • 15hp/credits
Swedish University of Agricultural Sciences, SLU
Department of Plant Breeding
Horticultural Science Program



Finding the physiological function of the enzyme GPCAT.

– A gene expression study using Arabidopsis plants with silenced GPCAT gene

Ida Hallström

Supervisor: **Ida Lager, SLU, Department of Plant Breeding**
Examiner: Åsa Grimberg, SLU, Department of Plant Breeding

Credits: 15
Level: G2E
Course title: **Independent project in Biology, Bachelor thesis**
Course code: **EX0854**
Programme/education: Horticultural Science program
Course coordinating dept.: Department of Plant Protection Biology

Place of publication:
Year of publication: **2022**
Cover picture:
Title of series:
Part number:
ISSN:

Keywords: enzyme GPCAT, gene expression, glycerophosphocholine acyltransferase, GPCAT, PC recycling pathway, physiological function, sphingolipids

Swedish University of Agricultural Sciences
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Plant Breeding

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file.

If you are more than one author you all need to agree on a decision. Read about SLU's publishing agreement here: https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Abstract

The aim of this project was to study the enzyme Glycerophosphocholine Acyltransferase (GPCAT) and try to find its physiological function. The biochemical reaction that the enzyme GPCAT catalyses is known, but its physiological function, e.g. why and when plants need the enzyme GPCAT, is unknown. To investigate the physiological function, *Arabidopsis* plants with (wild type) and without (knockout 19) the gene encoding for the enzyme GPCAT were cultivated and then exposed to abiotic stresses. The gene expression of six genes were studied by Q-RT-PCR and differences in gene expression was calculated by the Pfaffl method. The three genes that were associated with Sphingolipids (delta-9 acyl-lipid desaturase 2, very-long-chain 3-oxoacyl-CoA synthase and sphinganine C4-monooxygenase 1) showed higher gene expression in the knockout 19 plants than the wild type in the cold treatments. This implies that the silencing of GPCAT gene influences the genes in sphingolipids synthesis in the cold treated plants. Therefore, GPCAT might be involved in the regulation of lipids, with focus on sphingolipids, in cold stress.

Table of contents

Abbreviations

GPCAT	Glycerophosphocholine Acyltransferase
KO	Knockout 19
LPCAT	Lysophospatidylcholine Acyltransferase
PC	Phosphatidylcholine
WT	Wild type

1. Introduction

1.1. Membrane and lipids

Around eukaryotic cells and organelles are plasma membranes that consist of lipids and proteins (Yu et al., 2021, Bowsher et al 2008). The plasma membrane forms boundaries between cells and organelles and can by the help of transport proteins control the flow of metabolites through the membrane. Therefore, organelles can have different inner environments to optimize specific reactions. Most membrane lipids consist of a glycerol head with attached fatty acid chains, also called acyl groups. The membrane lipids are arranged in two layers with their hydrophilic heads outward and their hydrophobic fatty acid chains towards each other. This membrane organisation is called bilayer or the fluid mosaic model. The glycerol head can be composed of different polar groups and the fatty acids can vary in length and be saturated or unsaturated. The major categories of lipids are glycolipids, sphingolipids, and sterols.

As there are many varieties of lipids and its components they vary in characteristics. Galactolipids and phospholipids are the major lipid groups found in the membranes of plant cells. Phosphatidylcholine (PC), a member of the phospholipids, is the most abundant lipid in the membrane of non-photosynthesising organelles.

Membranes constantly change as a response to environmental factors (Bowsher et al 2008, Stålberg et al., 2008, Liu et al., 2019). One of these factors are abiotic stress. For example, cold tolerant plans have shown to have more lipids with unsaturated fatty acid chains (Bowsher et al 2008). As unsaturated fatty acid chains have double bond this leads to more loosely packed lipids that can keep their fluidity in colder temperatures.

1.2. Enzymes and lipid assembly

To facilitate the synthesis and renewal of lipids specific enzymes are used. Enzymes are proteins that catalyse biochemical reactions (Taiz et al, 2015). They are involved in almost all reactions and greatly increase the speed of the reaction without fundamentally changing themselves. Acyltransferase is a group of enzymes that transfers acyl groups (fatty acid chains).

With the help of enzymes different lipids are assembled in various pathways. One of these pathways is the Kennedy pathway (Gibellini and Smith, 2010, Napier and Graham, 2010, Li-Beisson et al., 2013). In the Kennedy pathway triacylglycerol (TAG) is assembled through several steps. The first step in the Kennedy pathway is that an acyl group is attached to glycerol-3-phosphate (G3P) with the help of an acyltransferase called glycerol-3-phosphate acyltransferase (GPAT), resulting in the substrate lysophosphatidic acid (LPA). Then another acyltransferase (lyso-phosphatidic acid acyltransferase, LPAAT) attaches the second acyl group resulting in phosphatidic acid (PA). The enzyme phosphatidic acid phosphatase (PAP) removes a phosphate group resulting in diacylglycerol (DAG). The last step in the Kennedy pathway is another addition of an acyl group by the enzyme diacylglycerol acyltransferase (DAGAT) forming TAG, typically stored in seeds. (Figure 1).

Several other enzymes are also involved in the formation of TAG from DAG (Gibellini and Smith, 2010, Li-Beisson et al., 2013). The enzyme phosphatidylcholine diacylglycerol cholinetransferase (PDCT), rearrange the phosphatidic choline group and PC is formed (Figure 2). The enzyme PDCT can catalyse in both directions and transform PC to DAG. In this way acyl groups can be moved from DAG to PC and vice versa. The enzyme phospholipid diacylglycerol acyltransferase (PDAT) removes an acyl group from PC and add it to DAG thereby making TAG and lysophosphatidyl choline (LPC).

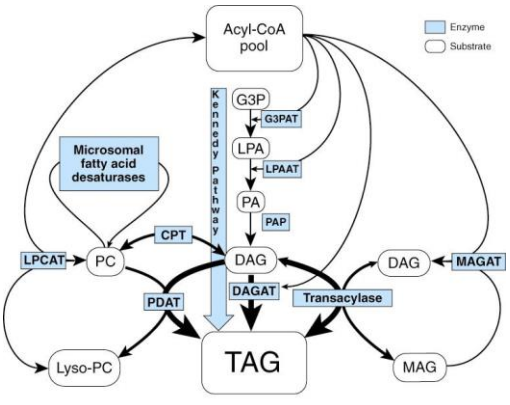


Figure 1. In the Kennedy pathway oil triacylglycerol (TAG) is assembled through several steps. Two first enzymes (GPAT: glycerol-3-phosphate acyltransferase and LPAAT: lyso-phosphatidic acid acyltransferase), attached an acyl group to the substrate (G3P: glycerol-3-phosphate and LPA: lysophosphatidic acid). Phosphatidic acid phosphatase (PAP) removes a phosphate group, the substrate phosphatidic acid (PA) becomes diacylglycerol (DAG). Diacylglycerol acyltransferase (DAGAT) add an acyl group forming TAG. (Picture: Naiper JA. 2007. Annu Rev. Plant Biol. 58:295-319)

From the substate LPC, lysophospatidylcholine acyltransferase (LPCAT) removes the other acyl group making glycero-3-phosphocholine (GPC). GPC have no acyl groups attached. The enzyme glycerophosphocholine acyltransferase (GPCAT) transfers an acyl group to GPC forming LPC and the enzyme LPCAT then attaches the second acyl group and PC is reformed. This way the acyl groups of PC can be changed (Figure 2).

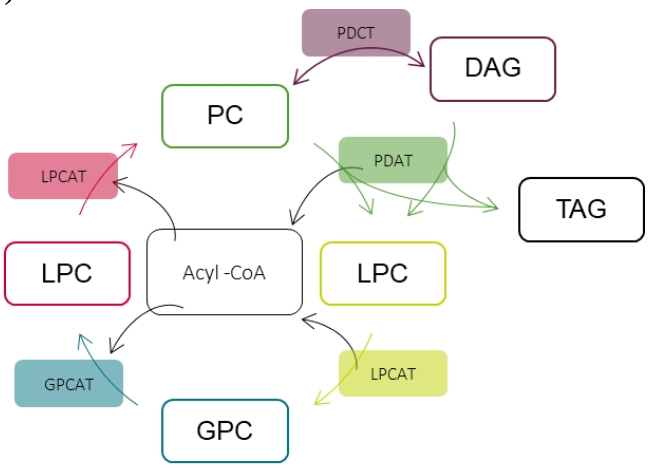


Figure 2: PC recycling pathway. With the help of different enzymes, the acyl groups in PC can be exchanged. Abbreviations: TAG: Triacylglycerol, DAG: Diacylglycerol, PDCT: phosphatidylcholine diacylglycerol cholinetransferase (also abbreviated to PDCT) PC: phosphatidylcholine, PDAT: LPC: lysophosphatidylcholine, LPCAT: lysophospatidylcholine Acyltransferase: GPC: glycero-3-phosphocholine, GPCAT: glycerophosphocholine Acyltransferase, Acyl – CoA: acyl groups (fatty acids) bound to Coenzyme A. (Illustration: Ida Hallström)

Understanding how lipids and its acyl groups is assembled is an important part in oil crop production and breeding. Oil in plant seeds consist mainly of TAG and have a variety of uses e.g. human consumption, biodiesel, soap, and pharmaceuticals (Dyer et al., 2008). The fatty acid composition of the different plant oils is very important for its end-use and differs a lot between plant species. Therefore, it is very important to understand the factors that control the lipid assembly and the final TAG fatty acid composition. With the rising concerns for the environment and the impact of using fossil fuel, the demand for oil from renewable resources such as oilseed crops are increasing.

1.3. Arabidopsis as a model plant

Arabidopsis thaliana has long been used as a model plant in genetic research (Gepstein and Horwitz, 1995, Anon 2002). The model plant has a relatively small genome size therefore making it easy to map and edit genes. It is also fast growing and small in size and the whole genome is documented making it advantageous for research.

1.4. GPCAT and gene selection

GPCAT was first identified in yeast, and by using sequence homology the enzyme was later identified in *Arabidopsis* (Głąb et al., 2016). The biochemical reaction that GPCAT catalyses is known, but its physiological function, e.g. why and when plants need the enzyme GPCAT is unknown. A common way of studying genes with an unknown function is to silence them in a model organism. There are several ways to silence a gene, one way is to use CRISPR/Cas9 to induce a mutation in the gene, thereby making the encoded protein non-functional. In that way plants without the gene can be compared to wild type plants and the physiological function of the gene of interest might be elucidated. Moreover, the plants can be subjected to an array of stresses, such as different climates, and thus study how the gene expression changes due to said stress.

In the same study (Głąb et al., 2016), the authors investigated the Membrane-based Interactome Network Database (M.I.N.D). MIND is a database for protein-protein interactions for *Arabidopsis* membrane proteins. The study found four protein-protein interactions for *Arabidopsis* that interacts with GPCAT. The genes encoding three of these proteins were selected to be tested in this project and are all associated with sphingolipids. From the PC recycling pathway, the gene coding for LPCAT 1 and LPCAT 2 was selected. GPCAT was only tested in the wild type plants.

Genes selected for this experiment:

- From PC recycling pathway
    - GPCAT
    - LPCAT 1
    - LPCAT 2
- Sphingolipid (Głąb et al., 2016)
    - Delta-9 acyl-lipid desaturase 2
    - Very-long-chain 3-oxoacyl-CoA synthase
    - Sphinganine C4-monooxygenase 1

1.5. Gene expression, Q-RT-PCR, and calculations

A way to measure the gene expression is to do a real time quantitative reverse transcription polymerase chain reaction (Q-RT-PCR or qPCR). In Q-RT-PCR, cDNA is used, cDNA is transcribed from mRNA as cDNA is a more stable and not as easily degraded. From the Q-RT-PCR a cycle threshold (CT) value is obtained. The CT value indicates the number of cycles before the sample crosses the threshold for the fluorescent signal. The lower the CT value is the higher amount of cDNA the sample contains; fewer cycles are required to reach the threshold i.e., the higher the gene is expressed.

To compare the gene expressions between the different samples, a housekeeping gene is used as a reference to normalise mRNA levels between the samples (Pfaffl, 2001). A housekeeping gene is defined as a gene that are expressed in all cells of an organism and codes for basic cellular functions. A good reference gene should also have the same CT value in all samples, independent on which tissue is used or what treatment that has been applied to the samples. It is often difficult to find stable reference genes.

To calculate the gene expression the Pfaffl method (Bradburn, 2018) uses the delta (average) CT value for the gene of interest and the delta of the housekeeping gene.

$$Gene\ expression\ ratio = \frac{(E_{GOI})^{\Delta Ct\ GOI}}{(E_{HKG})^{\Delta Ct\ HKG}}$$

E = Primer efficiency, assumed 100% = 2
GOI = Gene of interest
HKG = Housekeeping gene
ΔCt = Average CT value

To investigate significant difference between the gene expression wild type and knockout 19 in respective treatment a t-test was performed to analyse the results.

2. Aims

The aim of this project was to study the physiological function of the enzyme GPCAT in plants. This was done by comparing the expression of genes related to lipid metabolism in plants with (e.g. wild type) and without (knockout 19) the gene encoding for the enzyme GPCAT. Both normal and abiotic stress conditions were used in this study.

The null hypothesis states that no significant difference in the gene expression will be observed between the wild type and knockout 19 for each of the genes in respective climate condition.

3. Materials and Methods

3.1. Plant material and cultivation conditions

The seeds of two different *Arabidopsis thaliana* lines were sown and cultivated in controlled climate (Biotron, SLU-Alnarp) at 20°C, 60% humidity and day length of 16h, 260 µmol/m^2/s light (standard treatment). A wild type and one with a mutation where the gene encoding GPCAT previously had been silenced by the CRISPR/Cas9 method (here called knockout 19). On day 15 after sowing, the plants were transplanted into individual pots (18 plants of wild type, 18 plants of knockout 19). More plants than needed were cultivated and therefore plants were chosen randomly when transplanted. On day 35 after sowing, 6 plants each of wild type and knockout 19 were placed in a cold chamber at +3°C and a climate chamber at +30°C, both with full spectrum light (16h). After four days, leaf tissues were harvested and directly put in liquid nitrogen and then stored in a -80°C freezer. The Eppendorf tubes for RNA/ Q-RT-PCR were prepared with a couple of glass beads.

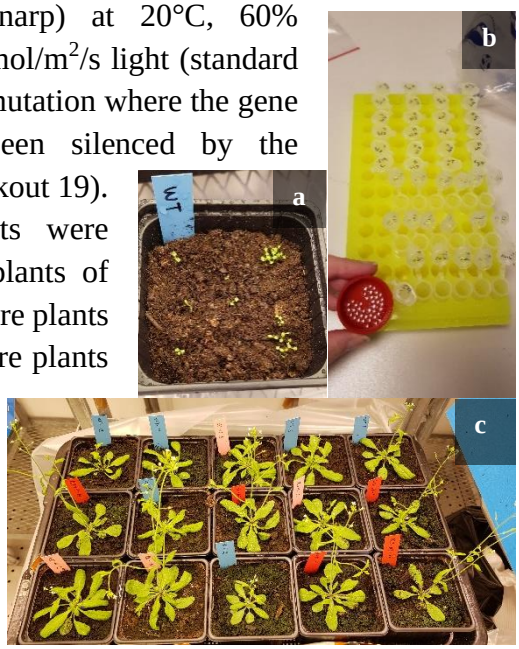


Figure 3. a: Arabidopsis thaliana, wild type plants before sorting, b: Eppendorf tubes being prepared for leaf sampling, c: Arabidopsis thaliana plants before heat and cold treatment. (Photo: Ida Hallström, 2021)

3.2. Primer design and selection for the selected genes

The primers for the genes (Table 1) were designed and selected with the help of the online program BLAST (National Center for Biotechnology Information). This program proposes primer pairs to the inputted gene in a selected plant. The primers were ordered from Invitrogen then diluted to 100µM and 10µM. Two primer pairs were orders for some of the genes.

The primers were first tested in a Q-RT-PCR to validate that they are functional and to select one of the duplicates. In all the duplicate, pair 1 performed better then pair 2, therefore pair 1 for all the duplicates was selected for the following Q-RT-PCR.

Table 1. The primers used in Q-RT-PCR reactions: name, their AGI number from TAIR Arabidopsis Genome Initiative (AGI) locus identifier and their sequence.

Name	*AGI number*	Sequence (5`->3`) (F=Forward, R=Reverse)
GPCAT	At5g35460	F-TTGGGTCATTTTCCTTCACCA R-TGAAAAACACGAGCCCAGGT
LPCAT 1	At1g12640	F-TCCTTTGGCTTCTCCTCCAAC R-CACTCATATAAAACACATGACAGCC
LPCAT 2 (Primer pair 1)	At1g63050	F-GTGCTGTCTGGCATGGACT R-GCATTGCCATTTTCGGAGGT
LPCAT 2 (Primer pair 2)	At1g63050	F-CATCCGTCGGTTTCATGGTTT R-GCTGAGAAGAAGCACAGCGA
Delta-9 acyl-lipid desaturase 2 (Primer pair 1)	At2g31360	F-CGTTACTTGGGGAATGGGGG R-TCTTCCAAGTTCGAGTGCCC
Delta-9 acyl-lipid desaturase 2 (Primer pair 2)	At2g31360	F-ACGTGGAGGATTTGAAGAGGC R-ATGCTGCTCCTACCCCCATT
Very-long-chain 3-oxoacyl-CoA synthase	At3g06470	F-TCCTCAAACCGATCACAGCC R-GTCGACGGGAAAGCAAATCG
Sphinganine C4-monooxygenase 1 (Primer pair 1)	At1g69640	F-CTTTTGTTCACGGTGACGGG R-TTGCCATGTGTCGAGGACTA
Sphinganine C4-monooxygenase 1 (Primer pair 2)	At1g69640	F-TTCTGTTGTGAAAGGTGTTCTTGT R-CTTCCCGTCACCGTGAACA

3.3. Q-RT-PCR

3.3.1. RNA Isolation

The samples from the plants were ground, using glass beads in the Eppendorf tubes and a Mixer Mill (Retch MM 400), for 45s at 30 Hz. The samples were kept cold with liquid nitrogen. The protocol PureLink® Plant RNA Reagent from Ambion® was used to isolate the RNA. In brief, the isolation reagent was added to frozen ground leaf tissue and through several steps of adding different solvents, mixing and cold centrifuging (to get rid of unwanted organic matter) a pellet with the isolated the RNA was formed in the Eppendorf tubes.

3.3.2. RNA Qualification

The RNA concentration and quality was measured with a spectrophotometer (Xpose, Trinean). The quality was also confirmed by running it on an agarose gel.

3.3.3. DNase Treatment

To remove genomic DNA from the isolated total RNA, a kit DNase 1, RNase-free from Thermo Scientific® was used.

Some of the DNase treated total RNA samples were kept and used as negative controls (minus reverse transcription control, -RT) when running Q-RT-PCR. This was to make sure that the RNA samples did not contain any genomic DNA contamination.

3.3.4. cDNA Synthesis

A kit from Thermo Scientific® (Maxima First stand cDNA synthesis kit for RT-qPCR) was used to transform the mRNA in the DNased total RNA to cDNA.

Both the cDNA and the previously DNase treated sample were then diluted with RNase free water to 500ng/200µL.

3.3.5. Q-RT-PCR

To add the fluorescence to the samples a kit from Thermo Scientific®, (maxima SYBR green/ROX qPCR master mix) was used. Then from Applied Biosystems™ the qPCR machine QuantStudio™ 3 was used with the three-step protocol (from the same kit) to obtain the CT values.

3.3.6. Housekeeping gene, replicates, and calculations

The housekeeping gene chosen in this study was actin.

Three biological replicates (i.e. three plants) were used and for each three technical replicates were performed. The average CT values and standard deviation were calculated for each sample (plant) and treatment. The purity of each sample was confirmed by a negative control (minus reverse transcription control, -RT). The DNase treated RNA were used as -RT.

To calculate the gene expression the Pfaffl method (Bradburn, 2018) was used.

4. Results

The gene expression for GPCAT was only tested in the wild type as the gene is silenced in the knockout 19 (the seeds for knockout 19, had been previously used and tested and the silencing of GPCAT confirmed). The CT average for each biological sample was calculated (Figure 4) and then also for each treatment (Figure 5). As housekeeping gene, actin was used for both wild type and knockout 19. The CT for the actin gene was calculated in the same manner (Figure 6 and 7). Then with the Pfaffl method the gene expression was calculated (Figure 8 and 9). The results showed that in wild type plants, the gene GPCAT was observed to be less expressed in cold treated plants compared to plants that were kept either in the Biotron (standard treatment) or at +30°C. To confirm this observation a t-test was used to calculate difference between the treatments (Table 2). A significant difference could be confirmed between the cold treatment and the heat treatment. Additionally, between the cold treatment and the standard (Biotron) treatment.

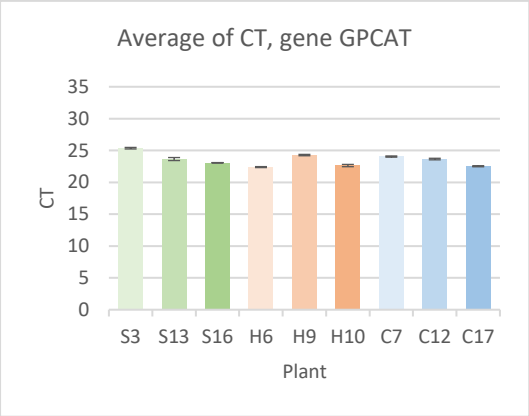


Figure 4. Average of CT values per wild type plant from Q-RT-PCR with the gene GPCAT. S=Biotron, standard treatment, H=heat treatment, C=cold treatment. Three technical replicates were performed for each plant. Error bars show standard deviation.

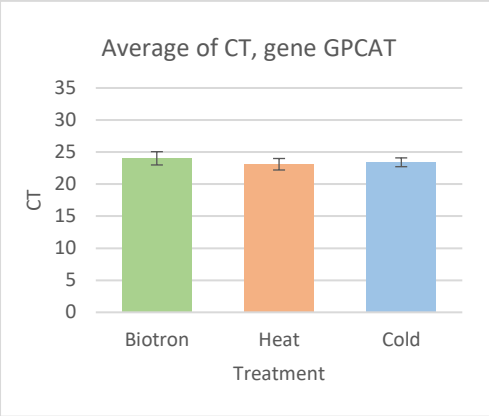


Figure 5. Average of CT values from Q-RT-PCR per treatment (Biotron=standard, heat, and cold treatment) of wild type plants with the gene GPCAT, n=3. Error bars show standard deviation.

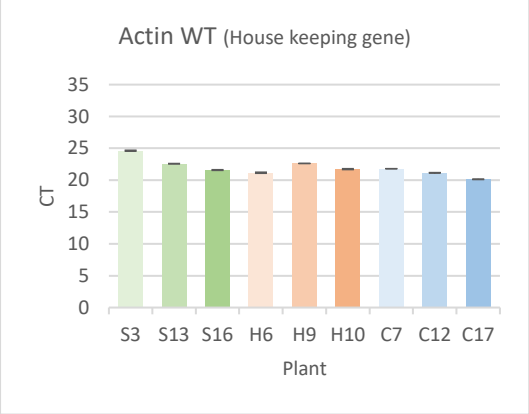


Figure 6. Average of CT values per wild type plant from Q-RT-PCR with the house keeping gene actin. S=Biotron, standard treatment, H=heat treatment, C=cold treatment. Three technical replicates were performed for each plant. Error bars show standard deviation.

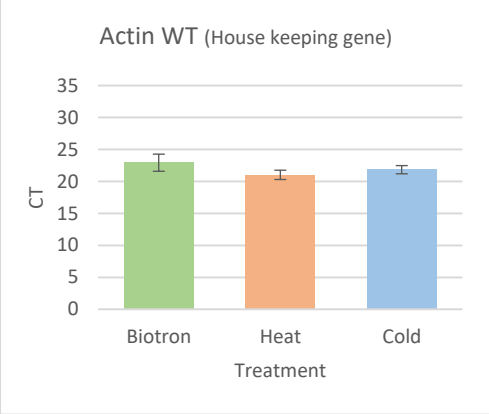


Figure 7. Average of CT values from Q-RT-PCR per treatment (Biotron=standard, heat, and cold treatment) of wild type plants with the house keeping gene actin, n=3. Error bars show standard deviation.

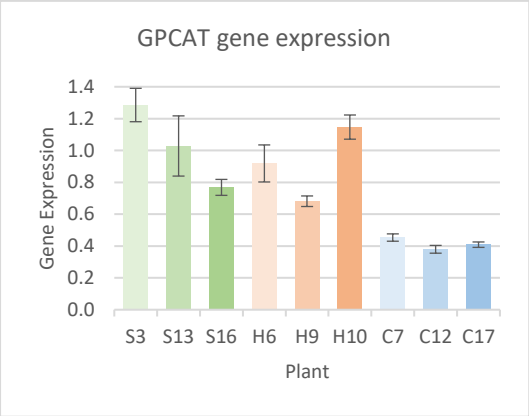


Figure 8 Average of gene expression in wild type of GPCAT per plant. S= standard treatment, H=heat treatment, C=cold treatment. Three technical replicates were performed for each plant. Error bars show standard deviation.

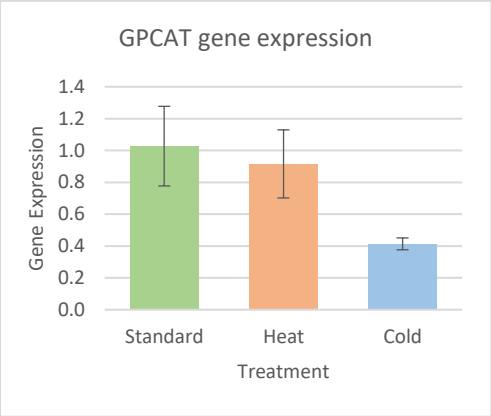


Figure 9. Average of gene expression (calculated by using the Pfaffl method) of GPCAT in wild type plants per treatment (standard, heat, and cold treatment). Error bars show standard deviation.

Table 2. P-values from of gene expression levels of GPCAT in wild type to determine significant differences between treatments ($p \leq 0,05$). A significant difference between cold and heat treatment and also between cold and standard, was observed.

T-test of gene expression (GPCAT)

	Standard	Heat	Cold
Standard	1,00	0,32	0,000002
Heat	0,32	1,00	0,000003
Cold	0,000002	0,000003	1,00

The expression levels of the housekeeping gene (actin) within the samples from the knockout 19 (Figure 10 and 11) showed no detectible values for sample S3 (Standard treated plant number 3). This was the same for all the tested genes. Therefore, the standard treated plants from knockout 19 only had 2 biological replicates (n=2).

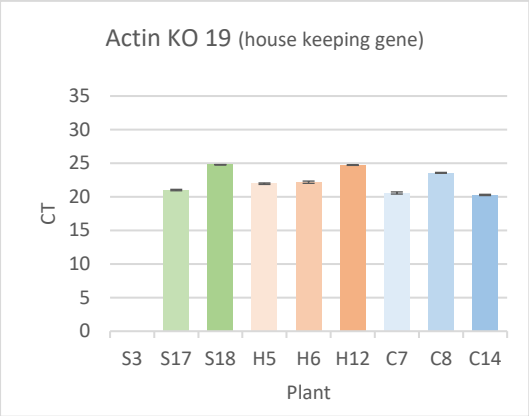


Figure 10. Average of CT values per knockout 19 plant from Q-RT-PCR with the house keeping gene actin S=standard treatment, H=heat treatment, C=cold treatment. Three technical replicates. S3: value was unreliable or undetectable. Error bars show standard deviation.

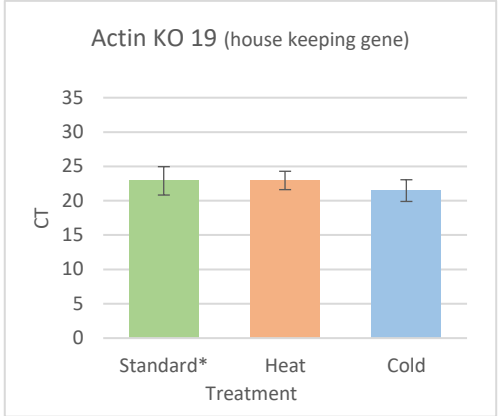


Figure 11. Average of CT values from Q-RT-PCR per treatment (Biotron=standard, heat, and cold treatment) of knockout 19 plants with the house keeping gene actin, n=3. Error bars show standard deviation.

4.1. LPCAT 1

Regarding the gene LPCAT 1, the results from the wild type were unreliable because of very high CT values and in several samples the gene expression was even undetectable, therefore no gene expression for the wild type and knockout 19 could be compared.

4.2. LPCAT 2

With the gene encoding LPCAT 2 the standard treated plants showed no significant difference in expression between the wild type and knockout 19 (Figure 12). In the heat-treated plants there was a significant difference between the two lines, where the gene LPCAT 2 in the knockout 19 was higher expressed than in wild type (Table 3). In the cold treatment a significant difference was also shown between the wild type and the knockout 19 where the LPCAT2 gene was higher expressed in knockout 19. But due to the high standard deviation in all the treatments, no definitive conclusion can be drawn.

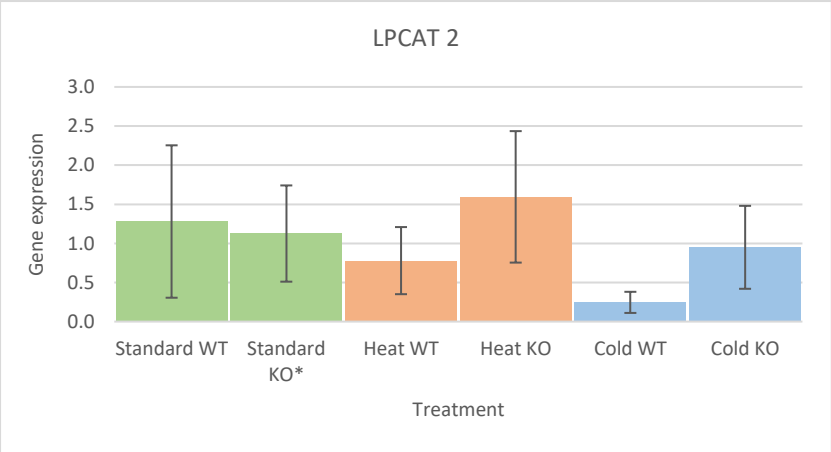


*Figure 12. Average value of gene expression of LPCAT 2 in wild type (WT) and knockout 19 (KO) per treatment (standard, heat, and cold treatment) n=3, *n=2. Error bars show standard deviation.*

*Table 3. P-values from t-test of gene expression levels of LPCAT 2 between wild type (WT) and knockout 19 (KO) to determine significant differences in the treatments ($p \leq 0,05$) n=3, *n=2. A significant difference was observed in the heat treatment and in the cold treatment.*

T-test of gene expression (LPCAT 2)

	Standard WT	Heat WT	Cold WT
Standard KO*	0,738085		
Heat KO		0,019661	
Cold KO			0,001392

4.3. Delta-9 acyl-lipid desaturase 2

The gene encoding delta-9 acyl-lipid desaturase 2, which was previously found to interact with GPCAT in the yeast-two-hybrid system (Głąb et al., 2016), was higher expressed in knockout 19 than in the wild type in the cold treated plants (Figure 13 and Table 4). No significant difference between the wild type and knockout 19 were observed in the treatments standard or heat. The highest expression of all the genes in this study was observed in the gene encoding delta-9 acyl-lipid desaturase 2 in knockout 19 in cold treatment.

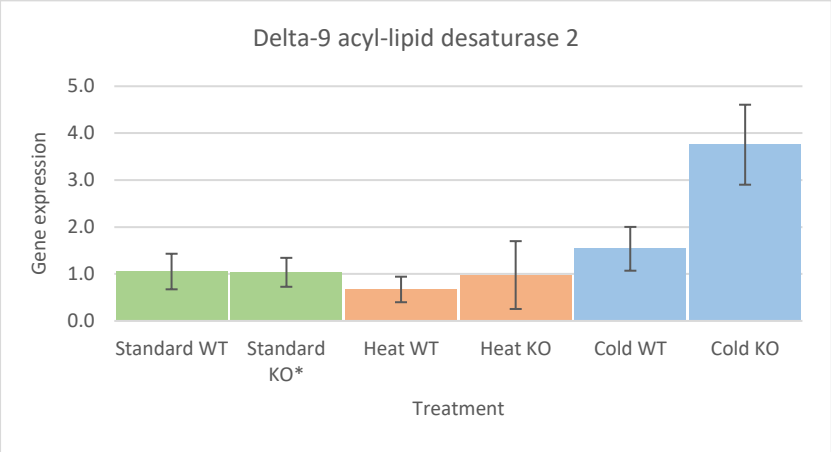


*Figure 13. Average value of gene expression of delta-9 acyl-lipid desaturase 2 in wild type (WT) and knockout 19 (KO) per treatment (standard, heat, and cold treatment) n=3, *n=2 Error bars show standard deviation.*

*Table 4 P-values from t-test of gene expression levels of delta-9 acyl-lipid desaturase 2 between wild type (WT) and knockout 19 (KO) to determine significant differences in the treatments ($p \leq 0,05$) n=3, *n=2. A significant difference was observed in the cold treatment.*

T-test of gene expression (Delta-9 acyl-lipid desaturase 2)

	Standard WT	Heat WT	Cold WT
Standard KO*	0,930035		
Heat KO		0,251150	
Cold KO			0,000004

4.4. Very-long-chain 3-oxoacyl-CoA synthase

Between the wild type and knockout 19, no significant difference was observed in the standard treatment in gene expression for the very-long-chain 3-oxoacyl-CoA synthase (Figure 14 and Table 5). No difference was observed in the heat treatment either. However, in the cold treatment a significant difference was observed between the wild type and knockout 19, where the knockout had a higher expression of the gene very-long-chain 3-oxoacyl-CoA synthase when compared to the wild type. Overall, the cold treated plants had a lower gene expression of very-long-chain 3-oxoacyl-CoA synthase than the standard and heat-treated plants.

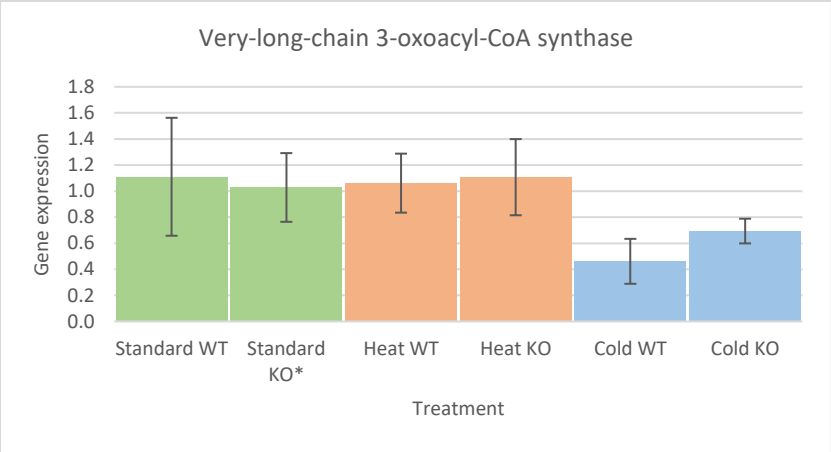


*Figure 14. Average value of gene expression of very-long-chain 3-oxoacyl-CoA synthase in wild type (WT) and knockout 19 (KO) per treatment (standard, heat, and cold treatment) n=3, *n=2 Error bars show standard deviation.*

*Table 5. P-values from t-test of gene expression levels of very-long-chain 3-oxoacyl-CoA synthase between wild type (WT) and knockout 19 (KO) to determine significant differences in the treatments ($p \leq 0,05$) n=3, *n=2. A significant difference was observed in the cold treatment.*

T-test of gene expression (Very-long-chain 3-oxoacyl-CoA synthase)

	Standard WT	Heat WT	Cold WT
Standard KO*	0,700242		
Heat KO		0,715748	
Cold KO			0,002725

4.5. Sphinganine C4-monooxygenase 1

No significant difference was observed in the standard treatment between wild type and knockout 19 regards to the expression of gene encoding sphinganine C4-monooxygenase 1 (Figure 15 and Table 6). In the heat treatment a significant difference was observed but since the standard deviation on the knockout 19 is high no definitive conclusion can be drawn. However, in the cold treatment a significant difference was observed in knockout 19 compared to wild type for the gene expression of sphinganine C4-monooxygenase 1. The lowest gene expression for the wild type was seen in the cold treatment.

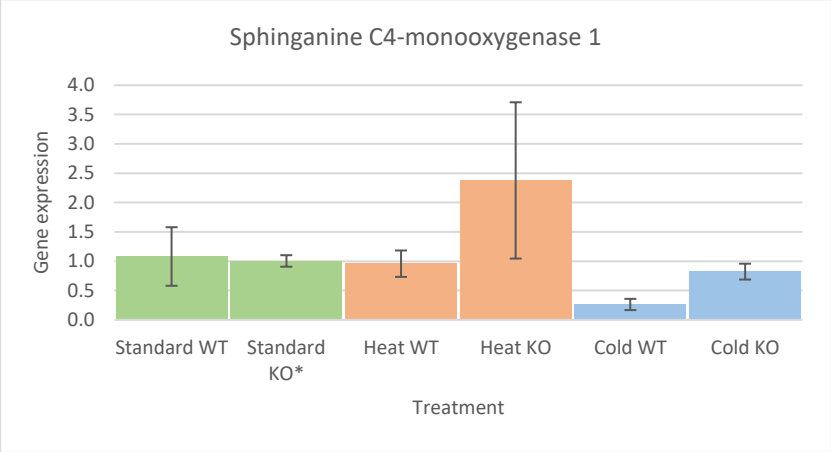


*Figure 15. Average value of gene expression of sphinganine C4-monooxygenase 1 in wild type (WT) and knockout 19 (KO) per treatment (standard, heat, and cold treatment) n=3, *n=2 Error bars show standard deviation.*

*Table 6. P-values from t-test of gene expression levels of sphinganine C4-monooxygenase 1 between wild type (WT) and knockout 19 (KO) to determine significant differences in the treatments ($p \leq 0,05$) n=3, *n=2. A significant difference was observed in the cold treatment.*

T-test of gene expression (Sphinganine C4-monooxygenase 1)

	Standard WT	Heat WT	Cold WT
Standard KO*	0,721759		
Heat KO		0,006178	
Cold KO			0,000000

5. Discussion

The aim of this project was to study the enzyme GPCAT and its physiological function. The biochemical reaction that GPCAT catalyse is known, but its physiological function, e.g. why and when plants need the enzyme GPCAT is unknown. A common way of studying genes with an unknown function is to silence them in a model organism. In that way plants without the gene can be compared to the wild type and the physiological function of the gene might be elucidated. In this study, the gene expression of six different genes encoding enzymes involved in plant lipid metabolism was analysed in a Q-RT-PCR.

The three genes that were associated with Sphingolipids (delta-9 acyl-lipid desaturase 2, very-long-chain 3-oxoacyl-CoA synthase and sphinganine C4-monooxygenase 1) showed higher gene expression in the knockout 19 plants than the wild type in the cold treatments. This implies that the silencing of GPCAT gene influences the genes in sphingolipids synthesis in the cold treated plants. Therefore, GPCAT might be involved in the regulation of lipids, with focus on sphingolipids, in cold stress. The null hypothesis, regards to genes that were associated with Sphingolipids, can therefore be rejected.

One of the functions of the membranes is to keep the cell in homeostasis, to do this the plant can alter the lipid composition (Bowsher et al 2008). When a plant is exposed to colder temperatures the lipids can exchange their acyl groups with acyls that have one or more double bonds. Unsaturated fatty acid chains have double bonds which leads to more loosely packed lipids that can keep their fluidity in colder temperatures, thus increasing that the plant tolerances to cold. Due to this it could also be interesting to do a fatty acid profile (FAP) especially regarding the cold treatment.

The results from LPCAT 1, were unreliable due to several high or undetected values in CT. When looking at LPCAT 2, the standard deviation was too high to draw any definitive conclusion. Due to the time limitation, it was not possible to repeat the experiment. To make sure that the results are valid the experiments should be repeated.

Factor that could affect the results in this study, were that all the climate parameters from the standard treatment could not be replicated in the heat and cold treatments due to the equipment. Therefore, the humidity and light quality can be an aspect that affected the result.

Moreover, it would have been interesting to test plants at a younger stage, since the plants we used here were fully developed. A younger plant would still increase the size of the leaves and thus we might have seen bigger changes. Also, it could be interesting to test the roots and therefore eliminate the aspect of the chlorophyll lipids in the samples. Another approach is to silence the gene or genes regarding the sphingolipids synthesis in *Arabidopsis* and examine the gene expression of GPCAT.

Finally, three samples is a start and an indication but to be more certain of the result further studies would need to be done and with more replications.

References

Bradburn, S, 2018, How To Perform The Pfaffl Method For qPCR Available at: //toptipbio.com/pfaffl-method-qpcr/ [Accessed 2021-11-29]

Bowsher, C., Steer, M., Tobin, A. (2008). Plant Biochemistry. 1st ed, New York US and Abingdon UK: Garland Science, Taylor & Francic Group, LLC, an informa buisness.

Dyer, J.M., Stymne, S., Green, A.G., Carlsson, A.S., 2008. High-value oils from plants. The Plant Journal 54, 640–655. https://doi.org/10.1111/j.1365-313X.2008.03430.x

Gepstein, S., Horwitz, B.A., 1995. The impact of Arabidopsis research on plant biotechnology. Biotechnology Advances 13, 403–414. https://doi.org/10.1016/0734-9750(95)02003-L

Gibellini, F., Smith, T.K., 2010. The Kennedy pathway—De novo synthesis of phosphatidylethanolamine and phosphatidylcholine. IUBMB Life 62, 414–428. https://doi.org/10.1002/iub.337

Głąb, B., Beganovic, M., Anaokar, S., Hao, M.-S., Rasmusson, A.G., Patton-Vogt, J., Banaś, A., Stymne, S., Lager, I., 2016. Cloning of Glycerophosphocholine Acyltransferase (GPCAT) from Fungi and Plants: A NOVEL ENZYME IN PHOSPHATIDYLCHOLINE SYNTHESIS*. Journal of Biological Chemistry 291, 25066–25076. https://doi.org/10.1074/jbc.M116.743062

Li-Beisson, Y., Shorrosh, B., Beisson, F., Andersson, M.X., Arondel, V., Bates, P.D., Baud, S., Bird, D., DeBono, A., Durrett, T.P., Franke, R.B., Graham, I.A., Katayama, K., Kelly, A.A., Larson, T., Markham, J.E., Miquel, M., Molina, I., Nishida, I., Rowland, O., Samuels, L., Schmid, K.M., Wada, H., Welti, R., Xu, C., Zallot, R., Ohlrogge, J., 2013. Acyl-Lipid Metabolism. arbo.j 2013. https://doi.org/10.1199/tab.0161

Lincoln Taiz, Eduardo Zeiger, Ian Max Møller and Angus Murphy, 2015 Plant Physiology and Development, Sunderland Massachusetts, Sinauer

Associates, Inc., 6th ed.
http://6e.plantphys.net/PlantPhys6e-appendix01.pdf

Liu, X., Ma, D., Zhang, Z., Wang, S., Du, S., Deng, X., Yin, L., 2019. Plant lipid remodeling in response to abiotic stresses. Environmental and Experimental Botany 165, 174–184.
https://doi.org/10.1016/j.envexpbot.2019.06.005

Napier, J.A., Graham, I.A., 2010. Tailoring plant lipid composition: designer oilseeds come of age. Current Opinion in Plant Biology 13, 329–336.
https://doi.org/10.1016/j.pbi.2010.01.008

National Center for Biotechnology Information
Available at: https://www.ncbi.nlm.nih.gov
[Accessed 2021-09-15]

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res 29, e45.

Stålberg, K., Neal, A.C., Ronne, H., Ståhl, U., 2008. Identification of a novel GPCAT activity and a new pathway for phosphatidylcholine biosynthesis in S. cerevisiae. Journal of Lipid Research 49, 1794–1806.
https://doi.org/10.1194/jlr.M800129-JLR200

UniProt
Available at: https://www.uniprot.org/
[Accessed 2021-09-15]

Yu, L., Zhou, C., Fan, J., Shanklin, J., Xu, C., 2021. Mechanisms and functions of membrane lipid remodeling in plants. The Plant Journal 107, 37–53.
https://doi.org/10.1111/tpj.15273

Acknowledgements

I would like to thank my supervisor, Ida Lager, for all her support and advice. My family and friends for their support and help.