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Biological Chemistry Bachelor's Program

Methylation of PP2A - Effects of heat stress on gene expression and phenotype of *Arabidopsis thaliana*

Sverre Schiølde Johansen (245318)

15.05.2021

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Abstract

The possible connection between protein phosphatase 2A (PP2A) and heat stress performance was explored in *Arabidopsis thaliana* by comparing wild type (WT) with a *lcmt1* knockout mutant. In healthy organisms the enzyme leucine carboxyl methyl transferase 1 (LCMT1) is responsible for methylating PP2A. A *lcmt1* mutant will not be able to methylate PP2A, and although the importance of methylation is poorly understood it has been shown to have a big effect under stress.

The plants were grown in Petri dishes and in soil, exposed to various lengths of heat stress and any phenotype differences were observed. Certain differences in gene response were also observed by the use of real-time PCR.

Results showed healthy growth, but with some differences in the mutant *lcmt1* compared with WT under normal condition. However, under stress WT would outperform *lcmt1* with the difference becoming more clear with increased stress.

The expression levels of different genes were explored, and differences were found. Quantification cycle (C_Q) and relative quantification (RQ) was examined, and especially values for heat shock protein 90-1 (HSP90-1) and iron-regulated transporter 1 (IRT1) stood out. Values were examined after 0 h, 1 h and 24 h of heat stress. The results were clearest after 24 h, where WT showed the strongest deviation from the control with strongly increased expression for HSP90-1 and strongly decreased expression for IRT1 compared with *lcmt1*.

Abbreviations

½ MS medium – Half strength Murashige and Skoog medium

APXS – Stromal ascorbate peroxidase, sAPX

cDNA – Complimentary DNA

C_Q – Quantification Cycle (C_T – Threshold Cycle)

DNA – Deoxyribonucleic Acid

EDTA – Ethylenediaminetetraacetic acid

FSD1 – Fe-superoxide dismutase 1

HSP90-1 – Heat shock protein 90-1

IRT1 – Iron-regulated transporter 1

lcmt1 – Leucine carboxyl methyltransferase 1 knockout mutant

LCMT1 – Leucine carboxyl methyltransferase 1 enzyme

PME1 – Protein phosphatase methylesterase 1

PP2A – Protein phosphatase type 2A

PP2A-C – Protein phosphatase type 2A catalytic subunit

RNA – Ribonucleic acid

RT-PCR – Real-Time Polymerase Chain Reaction (Revers Transcription Polymerase Chain Reaction)

RQ – Relative Quantification

SD – Standard Deviation

SE – Standard Error

UBC35 – Ubiquitin-conjugating enzyme 35

WT – Wild type

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1. Introduction

1.1 Protein phosphatases

In a phosphorylation cascade a signal is relayed with activated protein kinases to facilitate a cellular response. As long as the protein kinase is activated it will keep activating the next protein in the cascade by using an ATP and attaching a phosphate group to it. Protein phosphatases will inactivate the active protein by acting as a catalyst to remove the phosphate group from it. This process is called dephosphorylation. This makes up the phosphorylation-dephosphorylation system and act as a molecular switch to control activities in cells (Campbell N. A., et al., 2018).

1.2 Protein phosphatase type 2A – PP2A

Protein phosphatase type 2A is a protein complex consisting of three subunits. They are scaffolding (A), regulatory (B) and catalytic (C). Several variations of each exist. In *A. thaliana* there are three scaffolding, 17 regulatory and five catalytic variants making the theoretical number of different combinations 255 for the PP2A complex. The catalytic subunit PP2A-C is methylated by the enzyme Leucine carboxyl methyl transferase 1 (LCMT1) and demethylated by the enzyme Protein phosphatase methylesterase 1 (PME1). The full scope of the protein complex is still not fully defined. Figure 1.2.1 illustrates a homology model of one PP2A catalytic subunit created with Swiss-model (Creighton, et al., 2017; Lillo, et al., 2014).

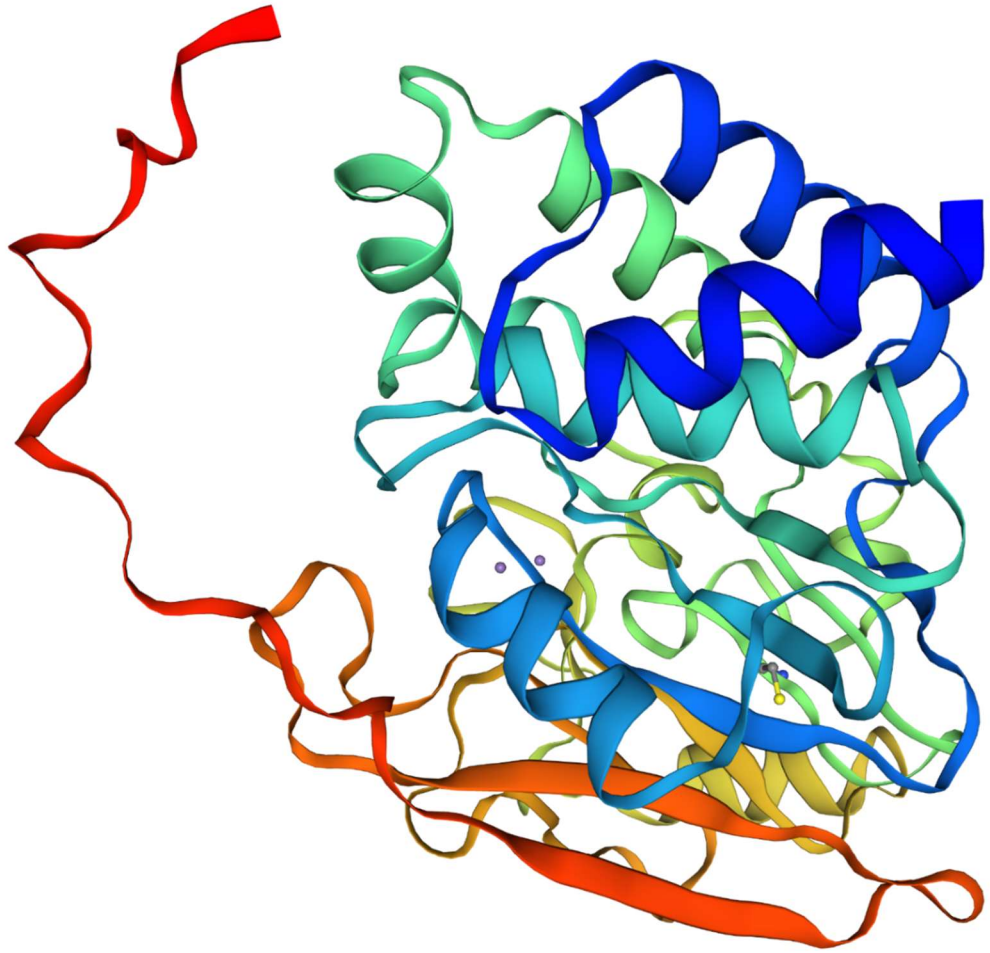


Figure 1.2.1: Homology model of one PP2A catalytic subunit from uniprot.org created with Swiss-model (uniprot.org; swissmodel.expasy.org, 2021, May 05)

1.3 *Arabidopsis thaliana* WT and *lcmt1*

With a knockout mutation in the LCMT1 enzyme, the mutant *lcmt1* will no longer be able to methylate PP2A-C. In *A. thaliana* this has shown *lcmt1* mutant to typically have less dense rosettes, earlier blooming flowers compared with WT and have a big impact during stress (Creighton, et al., 2017).

LCMT1 has been shown to be important and be preserved in both mammals, yeast and plants. For example, in mammalian cells it proved to be necessary for a normal progression through mitosis and knockout mutations in LCMT1 proved lethal in mice (Lillo, et al., 2014).

1.4 Stress

Plants prefer a certain amount of different abiotic environmental parameters to function optimally. They lack motility and must deal with whatever conditions their environment provide for them. Abiotic parameters include light, water, carbon dioxide, oxygen, soil nutrients, temperature and toxins. Deviation from optimal conditions will put stress on the plant and affect growth and reproduction. To compensate for this plants have evolved the ability to alter physiological and developmental processes to continue growing and reproducing. Under stress conditions perennial plants tend to adjust metabolism and developmental programs to optimise food storage until conditions improve. Annual plants however will more quickly enter the reproductive phase to ensure seed production even if it may result in fewer and smaller seeds (Taiz, et al., 2015).

Temperature stress affect membrane fluidity and can also block protein degradation. This can disrupt cellular functions. Heat stress can destabilize RNA and DNA secondary structures and lead to disruption of transcription, translation or RNA processing and turnover (Taiz, et al., 2015).

1.5 Real Time PCR

Polymerase Chain Reaction (PCR) has become a widely used molecular biology technique to amplify and detect RNA and DNA sequences because of its simplicity and speed. PCR is highly sensitive and requires little template and only a few hours to amplify a sample. The process uses DNA, so RNA is converted to complimentary DNA (cDNA) beforehand using revers transcriptase. This also has the added benefit of making the sample more stable since RNA is fragile and easily can degrade (Neidler, 2017).

In Real-time PCR the repeated steps of denaturation, annealing and elongation at different temperatures to amplify the DNA is performed using fluorescent molecules. The amount of fluorescence is measured in real-time to determine quantification of the amplified DNA (Neidler, 2017).

In the real-time PCR process using TaqMan gene expression assays temperature is first raised to denature the double stranded cDNA. When the temperature drops it allows for specifically designed forward and reverse primers together with a probe with a quenched dye to anneal to the denatured DNA. The probe is designed to bind to the 5’ end, and the primers synthesize new DNA from the 3’ to the 5’ end. When the primers reach the probe, the dye is released. For every cycle more dye is released increasing the intensity of fluorescence (Thermo Fisher Scientific, n.d.).

Quantification cycle (C_Q) is the cycle point where the fluorescence of the sample supersedes background noise. This is the number of cycles to detect a signal. A low number indicate strong expression of the gene in question and a high number indicate the opposite. The values are usually

normalized using the Delta-Delta C_Q method by comparing them with values of a gene known to not change much. In this thesis ubiquitin (UBC35) was used (TAIR, Arabidopsis.org, 2015), and Delta-Delta C_Q is the Relative Quantification (RQ) (Oswald, 2020).

The genes used in the analysis were APXS, FSD1, HSP90-1, IRT1 and UBC35.
APXS, or stromal ascorbate peroxidase, sAPX is involved in different kinds of response to oxidative stress, response to reactive oxygen species and hydrogen peroxide breakdown. It enables L-ascorbate peroxidase activity, heme binding and peroxidase activity (TAIR, Arabidopsis.org, 2015).
FSD1, or Fe superoxide dismutase 1 is involved in response to light intensity and ozone. It enables copper ion binding and superoxide dismutase activity (TAIR, Arabidopsis.org, 2013).
HSP90-1, or heat shock protein 90-1 is involved in cellular response to heat, chaperone-mediated protein folding, defence response to bacterium, protein folding and protein stabilization. It enables protein binding and unfolded protein binding (TAIR, Arabidopsis.org, 2015).
IRT1, or iron-regulated transporter 1 is involved in zinc ion transmembrane transport. It enables cadmium, iron, manganese and zinc ion transmembrane transporter activity (TAIR, Arabidopsis.org, 2021).
UBC35, or ubiquitin conjugating enzyme 35 is involved in postreplication repair, protein K63-linked ubiquitination and protein polyubiquitination. It enables protein binding, ubiquitin conjugating enzyme activity and ubiquitin-protein transferase activity (TAIR, Arabidopsis.org, 2015).

1.6 Aim

The aim for this thesis is to map effects heat stress has when the methylation levels of PP2A is low both phenotypically and regarding gene expression.

2. Material and Method

2.1 Hoagland Nutrient Solution (Hoagland & Arnon, 1950)

Using dry chemicals concentrated stock solutions were prepared firstly according to table 2.1.1 and 2.1.2 and stored in a cool and dark place. The stock solutions were made in one litre batches with distilled water. The stock solutions were then used to make a 10x concentrate for more convenient access. The 10x concentrate was then diluted with regular tap water to make the final 1x solution used in the experiments. Any final Hoagland 10x and 1x solutions not used immediately were also stored in a dark place.

Table 2.1.1: Overview for making the Hoagland nutrient solution and Hoagland stock solution.

	g/mole	g/L stock solution	1x solution	10x solution	Final concentration
KH_2PO_4 (1M)	136.086	136.086	1 mL	10 mL	1 mM
KNO_3 (1M)	101.10	101.10	5 mL	50 mL	5 mM
$Ca(NO_3)_2 \times 4H_2O$ (1M)	236.15	236.15	5 mL	50 mL	10 mM NO_3^- 5 mM Ca^{2+}
$MgSO_4 \times 7H_2O$ (1M)	246.48	246.48	2 mL	20 mL	2 mM
Fe-EDTA 1%	367.047	10	1 mL	10 mL	27 µM
Micronutrients			1 mL	10 mL	

Table 2.1.2: Overview for making the 1000x micronutrient stock solution for Hoagland Solution.

	g/mole	g/L stock solution	1000x solution concentration	1x solution concentration
H_3BO_3	61.83	2.86	46 mM	46 µM
$MnCl_2 \times 4H_2O$	197.91	1.81	9 mM	9 µM
$CuSO_4 \times 5H_2O$	249.677	0.089	0.36 mM	0.36 µM
$ZnSO_4 \times 7H_2O$	287.56	0.22	0.76 mM	0.76 µM
$Na_2MoO4 \times H_2O$	241.95	0.029	0.12 mM	0.12 µM

2.2 Growing *Arabidopsis thaliana* in soil and pest control

For these experiments a mixture consisting of ¾ parts soil and ¼ part vermiculite was used. A batch was prepared at the onset of each round of experiments. Three parallels in 100 mm pots were prepared for both WT and the *lcmt1* mutant for each repetition of each experiment.

The soil used was unfortunately contaminated with fruit flies and fruit fly larvae and since the maturation rate of fruit fly larvae is directly linked to humidity and temperature, both parameters with high values in these experiments, the soil was treated with Confidor insecticide at the beginning of each experiment. One tray of 3x2 pots with soil was soaked with about 1.5 L of Hoagland with Confidor. The concentration of Confidor was 0.1 g/L Hoagland. After two months the level of fruit flies started to increase, and all plants were treated with Confidor again.

Water was every time to a high practical degree added to the tray and not directly to the soil.

2.3 ½ MS media

For all agar experiments in Petri dishes the ½ MS media with 1% sucrose based on the Murashige and Skoog methods (Murashige & Skoog, 1962) was used. Similarly to Hoagaland, a ½ MS media stock solution was made first in one litre batches with dry chemicals weighed according to table 2.3.1. The chemicals were added to distilled water and the volume was adjusted up to 1 L. The stock solutions were stored in a cool and dark room. The ½ MS media was made according to table 2.3.1. The stock solutions and sucrose were added to distilled water and the volume was adjusted up to 1 L. The pH was adjusted with KOH up to 5.8 and the agar was added. The medium was divided between two 0.5 L flasks and then autoclaved. To melt the medium before use, the flasks were heated in a microwave oven.

Table 2.3.1: Overview for making ½ MS stock solution and final ½ MS solution.

	Mm g/mole	g/L stock solution	Volume stock/L ½ MS	Final concentration
KNO_3	101.10	95	10 mL	9.4 mM
NH_4NO_3	80.04	120	6.5 mL	9.7 mM
$MgSO_4 \times 7H_2O$	246.47	37	5 mL	750 µM
KH_2PO_4	136.09	17	10 mL	1.3 mM
$CaCl_2 \times 2H_2O$	147.01	44	5 mL	1.5 mM
Fe/EDTA:			25 mL	
Na_2EDTA	372.2368	0.373		34 µM
$FeSO_4 \times 7H_2O$	278.0146	0.278		25 µM
Minor I:			5 mL	
$ZnSO_4 \times 7H_2O$	287.541	0.920		16 µM
H_3BO_3	61.83	0.620		50 µM
$MnSO_4 \times 4H_2O$	223.0618	2.230		5 µM
Minor II:			5 mL	
$Na_2MoO_4 \times 2H_2O$	241.95	0.025		0.5 µM
$CuSO_4 \times 5H_2O$	249.69	0.003		0.06 µM
$CoCl_2 \times 5H_2O$	237.93	0.003		0.06 µM
KI	166.00	0.083		2.5 µM
Sucrose			10 g	1%
Agar			7 g	0.7%
Water			Up to 1 L	

2.4 Surface sterilization of Arabidopsis seeds

Table 2.4.1: Chemicals needed for surface sterilization of Arabidopsis seeds.

Chemical	Quantity
Ca-hypochlorite	0.25 g
Tween	1 drop
Ethanol	15 mL
Distilled water	25 mL

The surface sterilisation solution was prepared according to table 2.4.1. A solution of Ca-hypochlorite (0.25 g) with tween (1 drop) was prepared with distilled water (25 mL). It was well shaken and left to settle. The solution (supernatant) was pipetted (1 mL) to a falcon tube with 96% ethanol (9 mL). In a sterile workbench the seeds were then filled into Eppendorf tubes approximately a quarter full. The ethanol/Ca-hypochlorite solution (1 mL) was added to the Eppendorf tubes, shaken and left for no more than 4 minutes. The supernatant was pipetted off and ethanol (1 mL) was added to wash away any remaining solution. This was repeated twice. The ethanol was pipetted off and the Eppendorf tubes with the seeds were left uncapped in the sterile workbench to dry over night. The next day they were closed and sealed with parafilm.

2.5 Growing *Arabidopsis thaliana* in Petri dishes

For all experiments in Petri dishes the square 120 mm dishes were used. They were filled with approximately 30 mL ½ MS media each in a sterile workbench and stored in a cool place until needed. Only the standard ½ MS media described in section 2.3 was used.

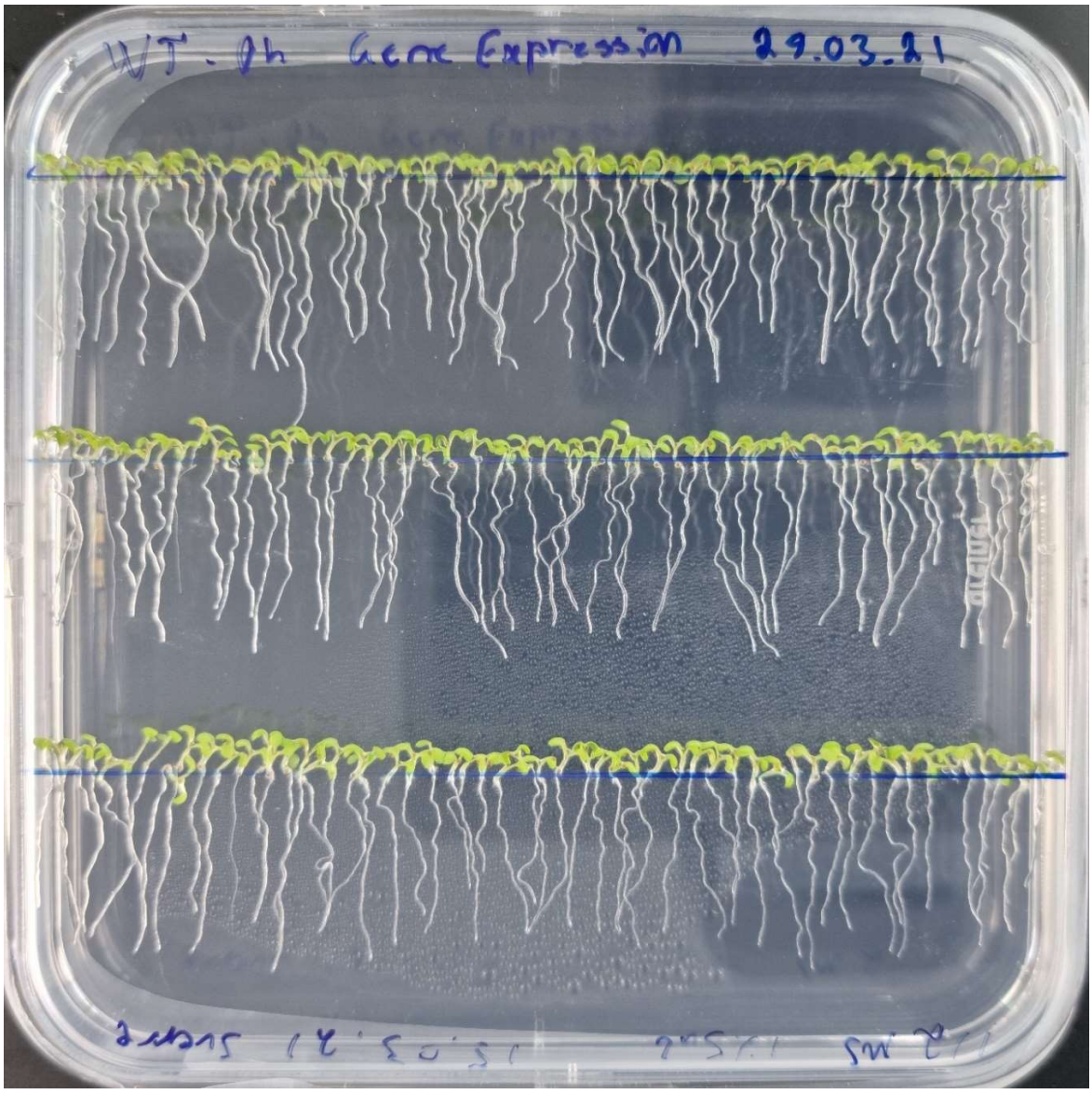


Figure 2.5.1: *Arabidopsis thaliana* seedlings after 5 d in the cultivation room. In a sterile workbench surface sterilized seeds were placed by hand using a sterilized wooden toothpick one seed at a time on the media in 120 mm Petri dishes, they were left in a cool and dark place for two nights and then put in an environment of artificial 16 h light/8 h dark cycle for an additional five nights.

Surface sterilized Arabidopsis seeds were in a sterile workbench placed by hand using a sterilized wooden toothpick one at a time on the ½ MS media in 120 mm Petri dishes. Wood was an excellent tool for this since its porous texture turned slightly sticky after being dipped in the agar media, but at the same time less sticky than the media itself. This made picking the seeds up easy and causing the seeds to usually prefer sticking to the dish when applied. The Petri dishes were then sealed with parafilm and put in a cool and dark place for two nights to ensure even germination. On the third day they were moved to an artificial environment of 16 h light/8 h dark cycle for an additional five nights. WT and *lcmt1* seeds were placed in separately marked Petri dishes.

For the heat treatment recovery experiment the most healthy and even seedlings were one week after being seeded transferred to new Petri dishes. Three parallels of the Petri dishes for each treatment (and control) were filled with five seedlings of WT and *lcmt1.* This was done in a sterile workbench and they were sealed with new parafilm afterwards. They were immediately following this exposed to the heat treatment for 0, 1 and 24 h at 37°C and moved back to the artificial environment for one more week after starting heat treatment. They were photographed before, immediately after and one week after heat treatment. The length the roots grew between the heat treatment and the end of the experiment were individually measured using ImageJ and the shoots were weighted five at the time as well at the end of the experiment. Since roots don't grow straight, they were measured with the freehand tool in ImageJ. The coastline paradox (Described by Lewis Fry Richardson, 1951) was considered and all measurements were attempted to be made with the same amount of accuracy.

For the gene expression analysis (figure 2.5.1) the seedlings were harvested immediately after the heat treatment. Because of this and because of the high number of seedlings needed to extract RNA they were not transferred to new Petri dishes and instead treated in the initial Petri dishes. Because of the limited growth of the seedlings after just one week about a hundred were needed for each treatment of both WT and *lcmt1,* in total about 600 seeds per parallel. For the RNA extraction, see section 2.6.

2.6 RNA extraction

For the RNA extraction process it was extra important to keep equipment and ingredients clean and uncontaminated. Clean uncontaminated samples of *Arabidopsis thaliana* from Petri dishes were used. The different samples of *A. thaliana* were flash frozen and kept frozen with liquid nitrogen and ground properly into fine dust with a pestle and mortar. The different samples were prepared and weighed as closely as possible to just under 100 mg each and kept in deep freeze at -80°C in marked individual Eppendorf tubes until the extraction process.

Before starting, buffer RLT and PureLink® DNase was prepared.

Buffer RLT was prepared in a fume hood with 10 µL β-Mercaptoethanol (β-ME) (toxic) per 1 mL Buffer RLT. It can be stored at room temperature for up to 1 month. The PureLink® DNase was prepared using according to table 2.6.1.

Table 2.6.1: Components to prepare PureLink® DNase.

Component	Volume (µL)
10X DNase I Reaction Buffer	8
Resuspended DNase (~3U/µL)	10
RNase Free Water	62
Final Volume	80

Buffer RLT and Buffer RW1 from the RNeasy® Plant Mini Kit (QIAGEN GmbH, Hilden, Germany).

PureLink® DNase mixture from On-column PureLink® DNase Treatment Protocol (Thermo Fischer Scientific, Inc, Waltham, MA, USA)

First part of extraction using the RNeasy® Plant Mini Kit:

- The Eppendorf tubes with the samples (90-100 mg) of finely ground plant material was each added the buffer RLT with β-ME (450 µL), vortexed vigorously and incubated at 56°C for 1-3 min.
- The lysate was transferred to a provided QIA shredder spin column (lilac) placed in a 2 mL collection tube and centrifuged for 2 min at 12 000 x g.
- The supernatant in the flow-through was transferred to a new microcentrifuge tube. It was important not to disturb the pellet of cell debris in the bottom.
- Ethanol (96%, 0.5 volume) was added to the lysate and mixed with pipetting, not centrifuging.
- The mixture (usually 650 µL) was transferred to a provided RNeasy spin column (pink), placed in a 2 mL collection tube, carefully closed and centrifuged for 15 s at 10 000 x g. The flow-through was discarded and the collection tube was reused.

Second part of extraction using On-column PureLink® DNase Treatment Protocol:

- Buffer RW1 (350 µL) was added to the spin column and it was centrifuged for 15 s at 12 000 x g. The flow-through was discarded and the spin column was inserted into a new collection tube.
- PureLink® DNase mixture (80 µL) was added directly to the spin column membrane and the samples were incubated at room temperature for 15 min.
- Buffer RW1 (350 µL) was added to the spin column and it was centrifuged for 15 s at 12 000 x g. The flow-through was discarded and the spin column was inserted into a new collection tube.
- Buffer RPE with ethanol (500 µL) was added to the spin column and it was centrifuged for 15 s at 12 000 x g. The flow-through was discarded. This was repeated once.
- The spin column was centrifuged for 2 min at 12 000 x g to dry the membrane with bound RNA. The collection tube was discarded and the spin column was inserted into a recovery tube.
- RNase-Free Water (25 µL) was added to the membrane and the spin column was incubated at room temperature for 2 min. The spin column with recovery tube was centrifuged for 1 min at 12 000 x g. This step was repeated once for a total of 50 µL RNase-free water with RNA.

2.7 cDNA synthesis

Table 2.7.1: Quantities of components used in the 2X RT master mix for cDNA synthesis per sample.

Component	Volume (µL)
10X RT Buffer	10
25X dNTP Mix (100 mM)	4
10X RT Random Primers	10
MultiScribe™ Revers Transcriptase	5
RNase Inhibitor	5
Nuclease-free H_2O	16
Total	50

To synthesize cDNA, all the components for the 2X RT master mix was first allowed to thaw on ice. They were then mixed according to table 2.7.1. 50 µL 2X RT master mix was then placed in separate PCR tubes. A mixture of RNA and nuclease-free H_2O with a volume of 50 µL and a concentration of 200 ng/µL per sample was prepared on ice as well. This was added to the PCR tubes with the 2X RT master mix bringing the total concentration to 100 ng/µL. The PCR tubes with a total volume of 100 µL were put in a Bio-Rad T-100™ thermal cycler which ran the program described in table 2.7.2 for cDNA synthesis. The synthesised cDNA was afterwards kept at -20°C for long term storage and later used for RT-PCR. (Applied Biosystems by Thermo Fischer Scientific, Foster City, CA, USA).

Table 2.7.2: The cDNA revers transcription program. It was important to set the correct reaction volume of 100 µL in the machine before running the program.

	Step 1	Step 2	Step 3	Step 4
Temperature (°C)	25	37	85	4
Time	10 min	120 min	5 min	∞

2.8 Expression analysis PCR

The real-time PCR analysis was performed using the TaqMan® Universal Master Mix II (Applied Biosystems by Thermo Fischer Scientific, Pleasanton, CA, USA) on a LightCycler® 96 (Roche Diagnostics GmbH, Mannheim, Germany).

Extra care was taken to make sure everything was clean and sterile. The volumes of each mixture were calculated and prepared beforehand. The volume per well is listed in table 2.8.1. Every sample had three parallels. The genes analysed is listed in table 2.8.2. The TaqMan® gene expression assays were allowed to thaw on ice, the needed amount was extracted, and the rest was put back in the freezer. The final concentration of the cDNA used in analysis needed to be within 1 to 100 ng in each well. This was calculated beforehand to be well within limits, but would usually be around 10 ng.

To the highest degree of accuracy the correct volumes were pipetted into each well and the plate was sealed with a plastic film. The plate was then centrifuged to get rid of bubbles and collect the content in the bottom before putting it in the thermal cycler for analysis.

The thermal-cycling program used for analysations by the LightCycler® 96 is listed in table 2.8.3.

Table 2.8.1: Overview of contents in each well in a Multiwell plate. Up to 96 wells in a run.

Components	Volume (µL) per reaction
TaqMan® Universal Master Mix II (2X)	10.0
TaqMan® Gene Expression Assay (20X)	1.0
cDNA template + RNase-free water	9.0
Total volume	20.0

Table 2.8.2: TaqMan® Gene Expression Assays used in the RT-PCR analysis.

Accession no.	Gene	TaqMan® Gene Expression Assay
AT4G08390	**APXS**, Stromal ascorbate peroxidase, sAPX.	At02210258_g1
AT4G25100	**FSD1**, Fe superoxide dismutase 1	At02238153_g1
AT5G25640	**HSP90-1**, Heat shock protein 90-1	At02320696_g1
AT4G19690	**IRT1**, Iron-regulated transporter 1	At02164076_gH
AT1G78870	**UBC35**, Ubiquitin-conjugating enzyme 35	At02612351_g1

Table 2.8.3: Thermal-cycling program on LightCycler® 96

Cycles	Temperature (°C)	Time	Step
1	95	10 min	Preincubation
45	95	10 s	2 Step Amplification
	60	30 s	
1	40	30 s	Cooling

3. Results

3.1 Growing *Arabidopsis thaliana* in Petri dishes

During the course of the work on this thesis, some laboratory work were temporarily suspended a few times due to the development of the Covid-19 situation. This was one factor that made experiment work and planning a challenge.

Another challenge was dealing with a few bad seed batches. Upon returning to laboratory work, progress was lost due to some batches of seeds not germinating properly. Another batch of seeds was also discovered to have been contaminated. Both these cases could possibly stem from under or over exposure to the chemicals in the surface sterilization process. Because time was a premium, one Petri dish was grown from each batch of seeds to determine its quality. Figure 3.1.1 showed a bad batch to be easy to spot when comparing with a good one. Healthy seeds of *Arabidopsis thaliana* showed an excellent rate of germination close to 100%, and very even growth rate as well. A contaminated Petri dish of seeds would easily contain one or dozens of mould colonies, and be easy to spot as well.

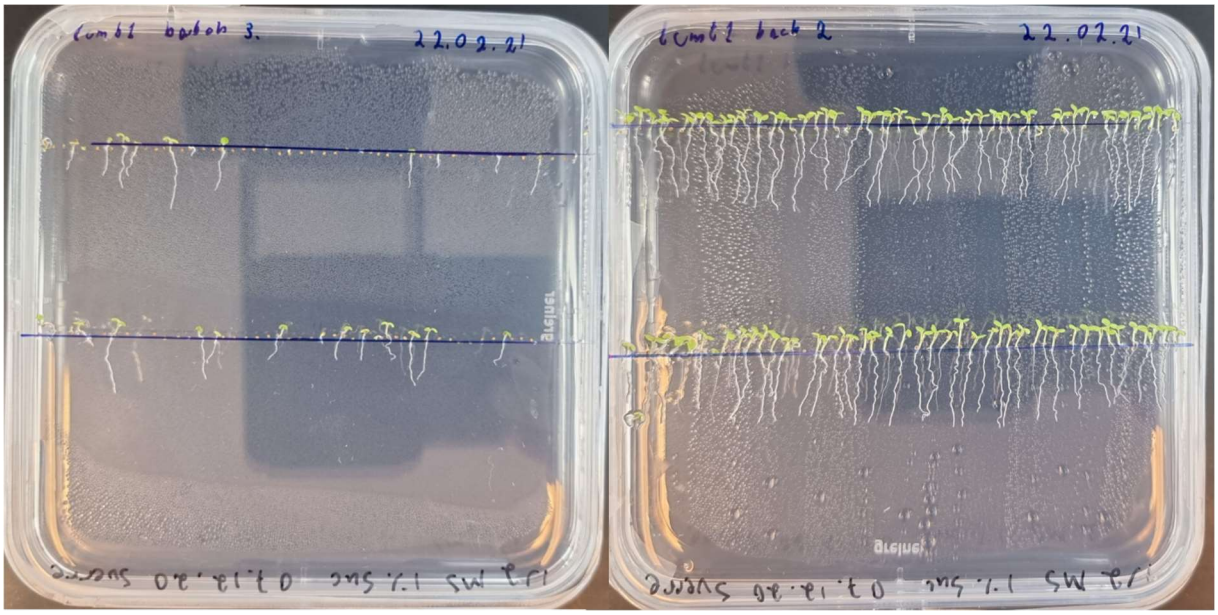


Figure 3.1.1: Comparing seed quality. A bad batch could easily be distinguished from a good one. Illustrated two samples of different batches of *lcmt1* after 5 d in an artificial environment of 16 h light/8 h dark cycle at approximately 22°C.

Normally seedlings in Petri dishes are allowed to grow for five days before exposed to an experiment. This was the case for both the recovery experiments and the gene expression experiments. However, for the gene expression experiments it was essential to preserve the state immediately after end of heat exposure. This meant that in order to get enough material for RNA extraction, close to 100 mg per sample, a large amount of seeds were needed. In fact, thousands of seeds were used for that purpose. Because of this it might be beneficial to use more mature seedlings in the future.

3.2 First trial soil experiment: Recovery from 18 hour heat stress at 37°C

The first trial soil experiment with monitored recovery from 18 h of heat stress at 37°C showed a clear difference in the phenotype between WT and *lcmt1* (figures 3.2.1 and 3.2.2). WT grew fuller thicker leaves before the start of the experiment and withstood the exposure better with stronger growth after compared to *lcmt1*.

Interestingly there was a gradient of increasingly deteriorating plants towards the back of the container. This might be caused because the fan in the heat chamber exposed towards the back blew the hot air directly at the container. Future experiments were conducted with the containers as far away from the fan as possible. The angle of the containers were kept unchanged because a heat gradient among both WT and *lcmt1* would still ensure equal conditions in contrast to exposing just WT or just *lcmt1* to the hot air flow.

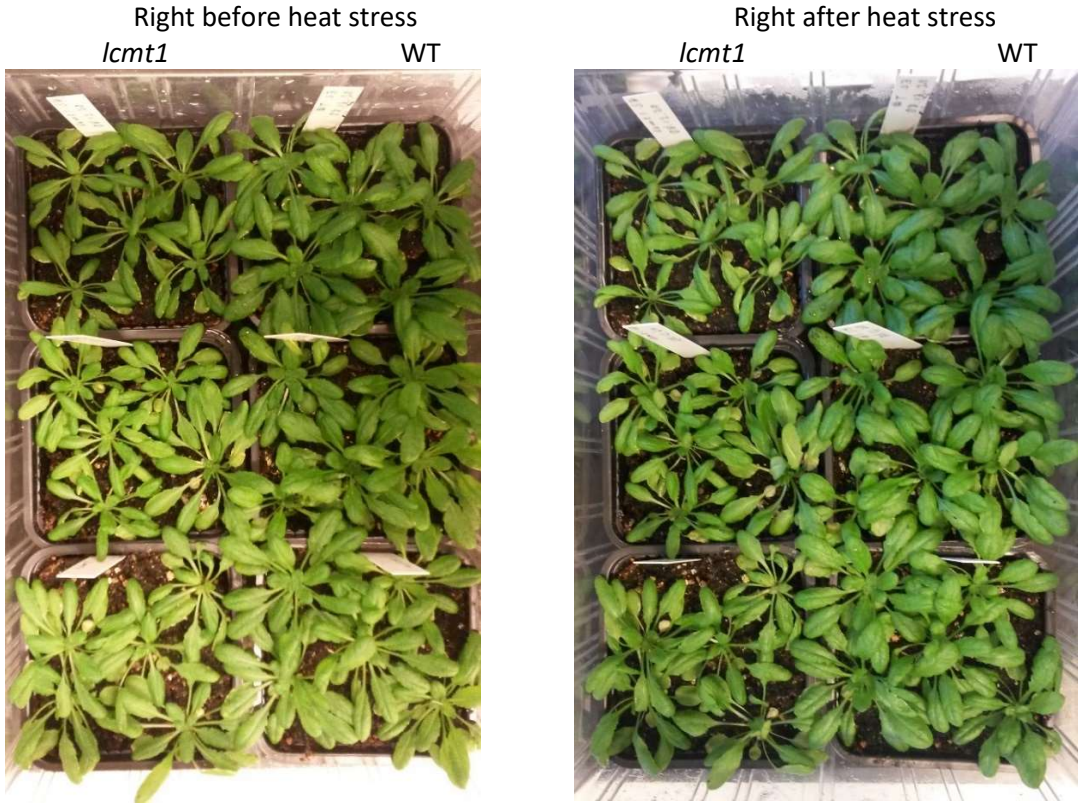


Figure 3.2.1: Heat stress experiment of *Arabidopsis thaliana*. The plants were grown in soil with a 12 h light and 12 h dark cycle and watered with a standard Hoagland nutrient solution. After five weeks the plants were heat stressed and monitored.

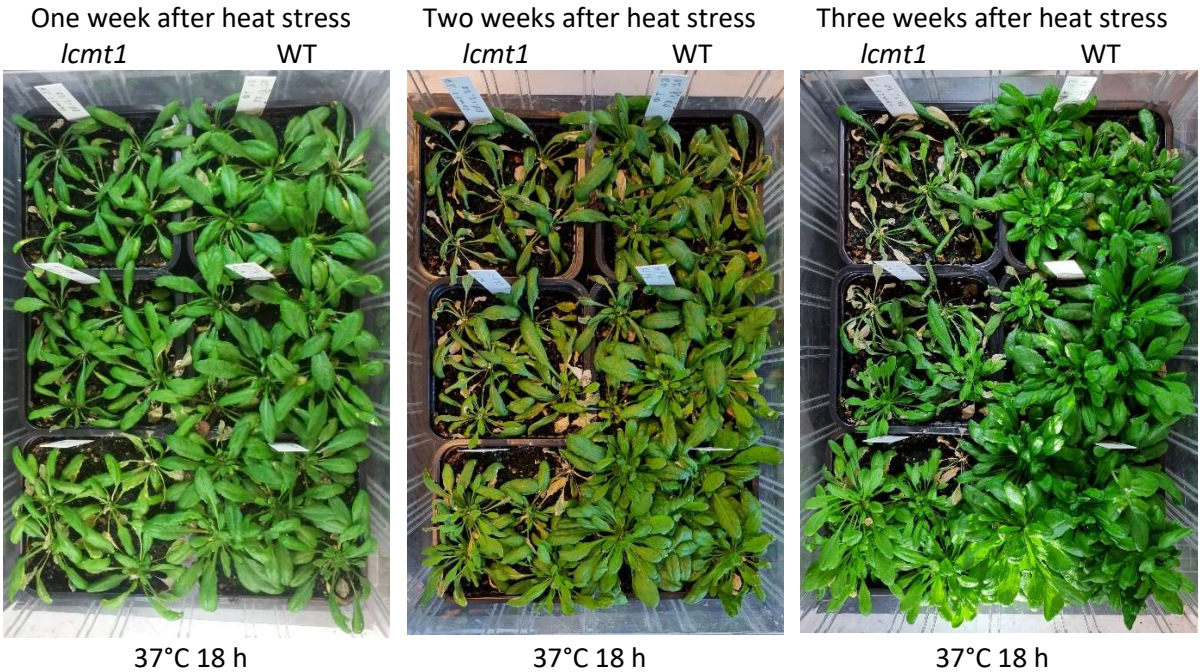


Figure 3.2.2: Heat stress experiment of *Arabidopsis thaliana*. Depiction of the follow up of the recovery process over three weeks.

3.3 Soil experiment: Recovery from 0, 8, 18 and 24 h heat stress at 37°C

In this experiment *Arabidopsis thaliana* WT and *lcmt1* were watered with standard Hoagland solution and grown in soil for five weeks in a controlled environment of 12 h light/12 h dark, but with some daylight from windows. They were then exposed to different lengths of heat at 37°C. Control with no exposure, 8 h in an open container and 18 h and 24 h in a closed container. The plants were kept for two more weeks, photographed and progress recorded.

Observations of the phenotype (figure 3.3.2) indicated WT grew broader thicker leaves with a darker green colour compared to *lcmt1.* In general the mutant *lcmt1* displayed a higher willingness to reproduce in the control and 8 h groups (figure 3.3.3 – 3.3.4) and were quicker to grow the central stem and blooming flowers on it, but with increased stress there was a tipping point in the 18 h group (figure 3.3.5) where the mutant *lcmt1* could not keep up with the WT who produced stems with blooming flowers the fastest. WT hardly produced any stems after two week in the control group, but an increased willingness to do so with increased heat stress (figure 3.3.1). Each of the three parallels contained three pots with five plants in each for both WT and *lcmt1*. The total number of plants were 45 for both WT and *lcmt1*. Figure 3.3.1 displayed the values as a percentage of the total number of plants.

In this experiment no plants recovered from exposure to 37°C for 24 h (figure 3.3.6).

No group in this experiment showed any sign of gradience of deterioration towards the back of the container. It could be coincidental in the first experiment, or keeping the containers towards the front of the heat chamber away from the fan might have been enough to cancel out the effect.

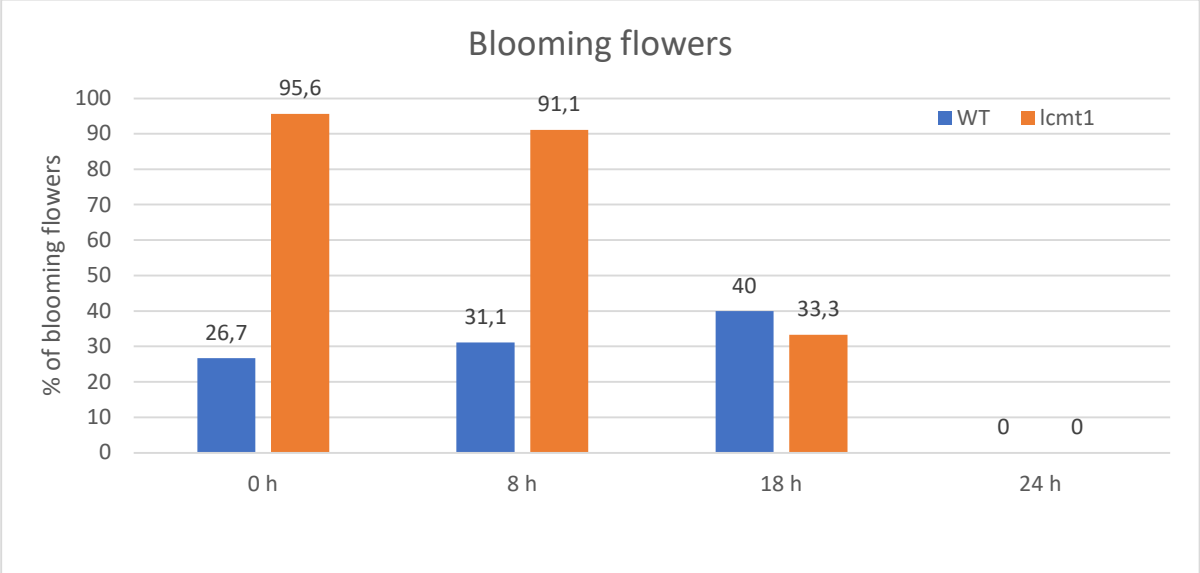


Figure 3.3.1: Mean blooming numbers displayed as a percentage of *Arabidopsis thaliana* WT and *lcmt1* after two weeks of recovery from heat exposure. The plants were exposed to different lengths of heat at 37°C. Control with no exposure (0 h), 8 h in an open container and 18 h and 24 h in a closed container.

Figure 3.3.2: The growth of *Arabidopsis thaliana* WT and *lcmt1* 8 h, 18 h and 24 h in soil before heat exposure. The plants were kept in a controlled environment of 12 h light/12 h dark, but with windows for the duration of the experiment. They were watered with a standard Hoagland solution and grown for five weeks before exposed to the heat treatment.

Figure 3.3.3: The growth of *Arabidopsis thaliana* WT and *lcmt1* control group in soil exposed to 0 h of heat. Illustrated progress at start of experiment, after one week and after two weeks of recovery. The plants were kept in a controlled environment of 12 h light/12 h dark, but with windows for the duration of the experiment. They were watered with a standard Hoagland solution and grown for five weeks before start of the experiment.

Figure 3.3.4: The growth of *Arabidopsis thaliana* WT and *lcmt1* in soil exposed to 8 h of heat in an open container. Illustrated progress directly after heat treatment experiment, after one week and after two weeks of recovery. The plants were kept in a controlled environment of 12 h light/12 h dark, but with windows for the duration of the experiment. They were watered with a standard Hoagland solution and grown for five weeks before exposed to the heat treatment.

Figure 3.3.5: The growth of *Arabidopsis thaliana* WT and *lcmt1* in soil exposed to 18 h of heat in a closed container. Illustrated progress directly after heat treatment experiment, after one week and after two weeks of recovery. The plants were kept in a controlled environment of 12 h light/12 h dark, but with windows for the duration of the experiment. They were watered with a standard Hoagland solution and grown for five weeks before exposed to the heat treatment.

Figure 3.3.6: The growth of *Arabidopsis thaliana* WT and *lcmt1* in soil exposed to 24 h of heat in a closed container. Illustrated progress directly after heat treatment experiment, after one week and after two weeks of recovery. The plants were kept in a controlled environment of 12 h light/12 h dark, but with windows for the duration of the experiment. They were watered with a standard Hoagland solution and grown for five weeks before exposed to the heat treatment.

3.4 First trial ½ MS heat treatment recovery experiment

The initial trial experiment showed that WT and mutant *lcmt1* would perform differently when exposed to heat stress. Figure 3.4.2 showed that the root lengths were very similar between the two in the control, but root growth would be severely impacted by 24 hours heat treatment at 37°C in WT, and in the mutant *lcmt1* growth would almost stop. WT would in general perhaps grow more branching of the roots compared to the mutant *lcmt1*. Also for some reason the plants in control parallel 3 grew visually thinner (figure 3.4.1) than the other two. This was consistent between both WT and the mutant *lcmt1* in parallel 3. Figure 3.4.3 showed a similar trend, but regarding shoot weight WT performed better under both conditions. However, shoot weight were severely reduced compared to the control after the heat treatment for 24 h. The difference in shoot weight between WT and the mutant *lcmt1* were not as clear in the heat treatment group compared to the clear difference in root length, at least not with such a small selection. Table 7.2.2 also show the weakest shoot weight in control parallel 3. It should be noted that the shoots were weighed five at the time in contrast to the root lengths that were measured individually.

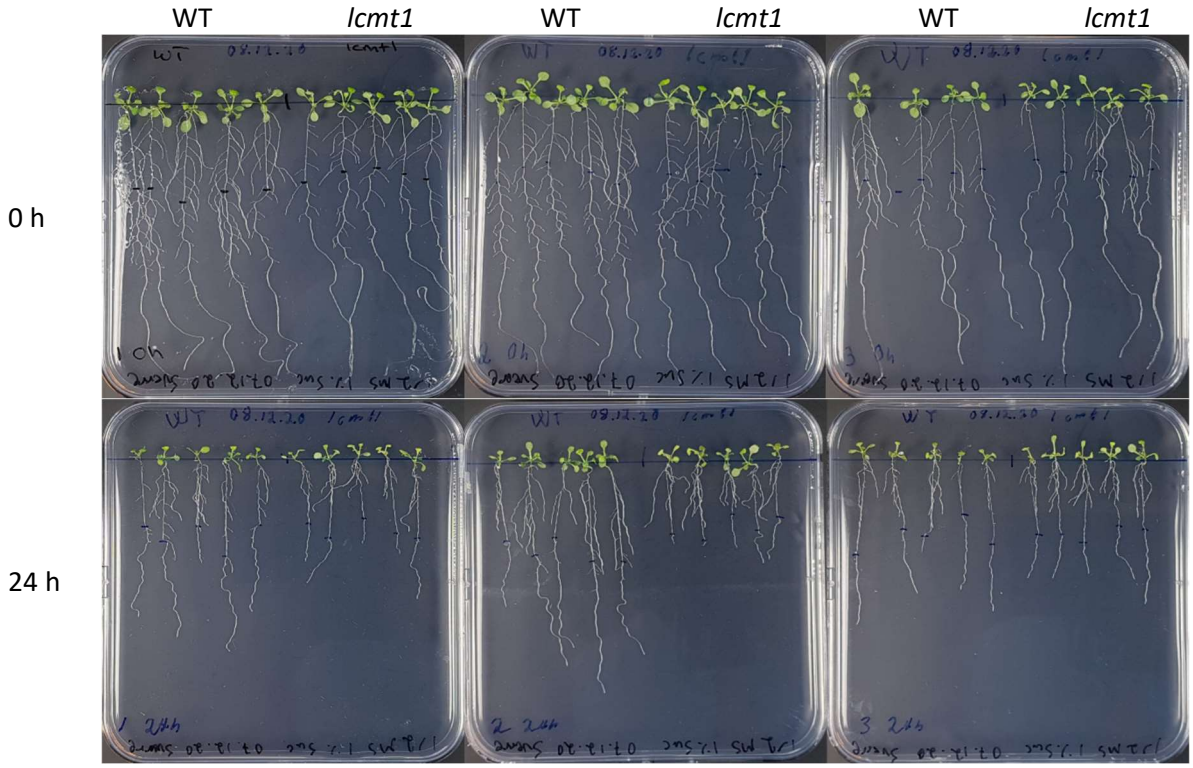


Figure 3.4.1: The trial heat treatment recovery experiment. The growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. The root length was marked immediately before start of heat treatment.

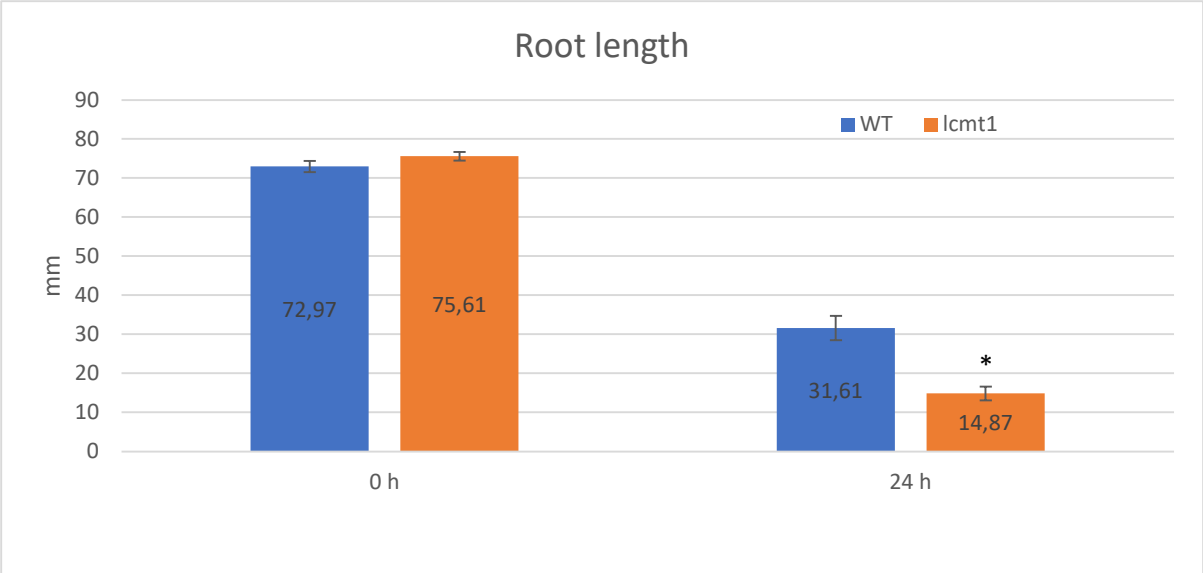


Figure 3.4.2: The mean root length growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. WT showed shorter growth than *lcmt1* in the control. After 24 hours both WT and *lcmt1* showed significant reduced growth, but *lcmt1* showed approximately half the growth of WT. n=8 for WT-0h and n=9 for all other groups, SE is shown as vertical bars. The value for 24h was significant (star), $p<0.05$.

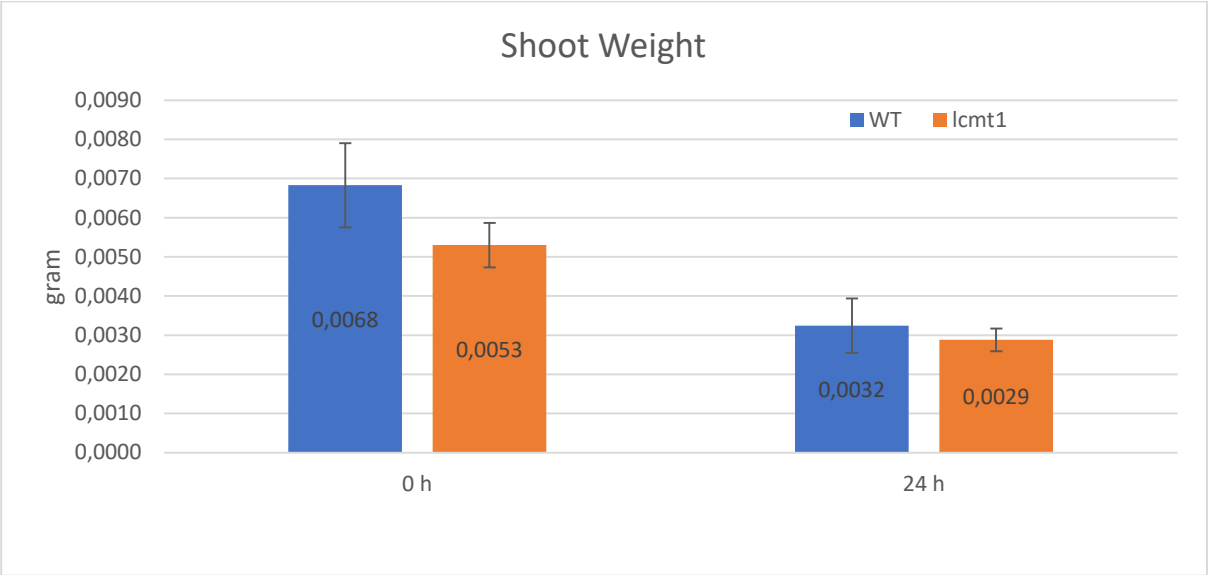


Figure 3.4.3: The mean shoot weight of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. WT showed increased growth compared to *lcmt1* under both conditions. After 24 hours both WT and *lcmt1* showed significant reduced growth. n=3, SE is shown as vertical bars. No values were significant, $p<0.05$.

3.5 ½ MS heat treatment recovery experiment 1: Root length and plant weight after one week

The first full ½ MS heat treatment experiment include a group heat treated for one hour in addition to the control and the 24 h group. This was to have data in tandem with the gene expression experiment who also tested for 0, 1 and 24 h. This experiment clearly showed that WT and *lcmt1* performed differently when exposed to heat stress. Figures 3.5.4 and 3.5.5 indicated an increased root length and plant weight in both WT and *lcmt1* in the 1 h group compared with the control, but a large reduction for both in the 24 h group.

The mean root length of *lcmt1* were longer than WT in the control and after one hour heat treatment, but were significantly reduced compared with WT after 24 h heat treatment. Figures 3.5.1, 3.5.2 and 3.5.3 all showed that even though *lcmt1* grew longer roots, WT in general had a lot more branching of the roots.

Figure 3.5.5 showed that even though *lcmt1* grew longer roots in the control and after 1 h heat treatment the weight of the plant would be less than WT in all groups, and significantly so in the 1 h and 24 h groups, $p<0.05$. It should be noted that the plants were weighed five at the time in contrast to the root lengths that were measured individually.

Figure 3.5.1 and 7.3.1 showed all plants of both WT and *lcmt1* in control parallel 1 plate 2 underperformed in comparison to all other controls and even the entire 1 h group as well. This was considered a clear anomaly, and the results of the experiment was recalculated with that plate omitted.

The modified results showed a much more even growth pattern when comparing the control with the 1 h group both in regard to root length and plant weight (figures 3.5.6 and 3.5.7). The values for root length were also significant in the modified control group.

The values of whole plant weight would include the clear difference in root volume between WT and *lcmt1*, but roots were wet and included a large surface area which made evaporation a problem and the values difficult to get accurate readings on.

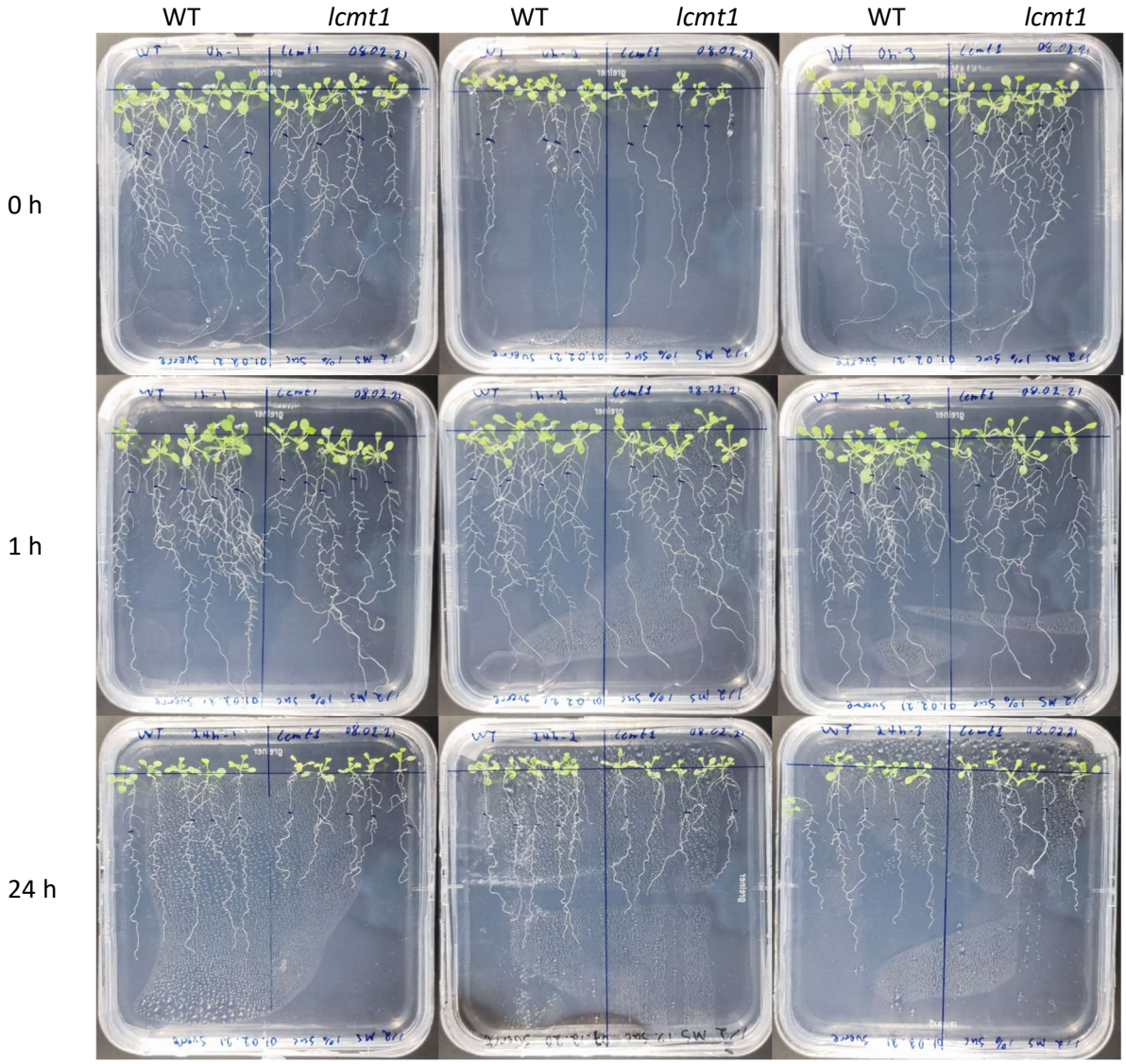


Figure 3.5.1: First parallel of heat treatment recovery experiment 1. The growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. The root length was marked immediately before start of heat treatment.

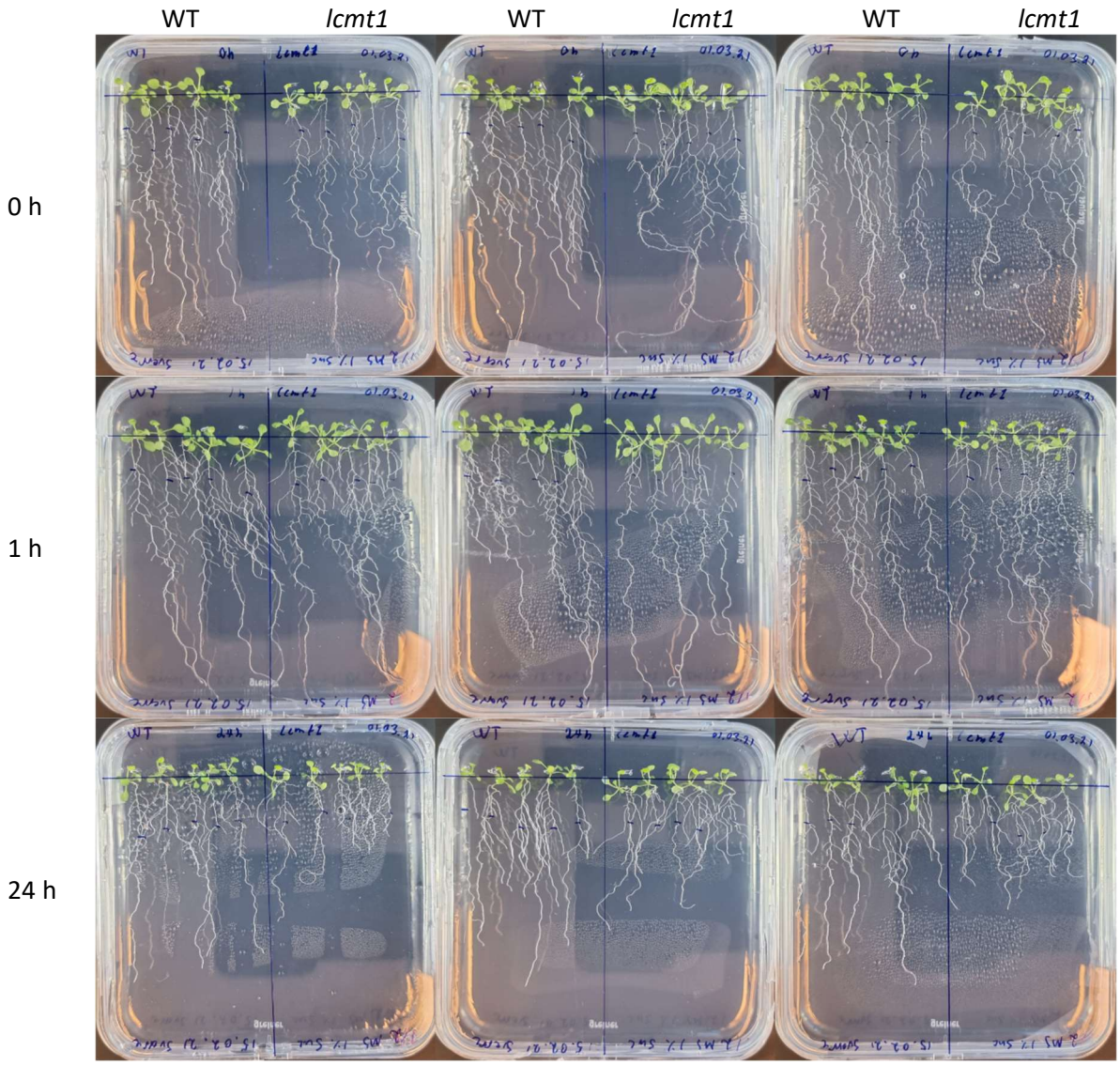


Figure 3.5.2: Second parallel of heat treatment recovery experiment 1. The growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. The root length was marked immediately before start of heat treatment.

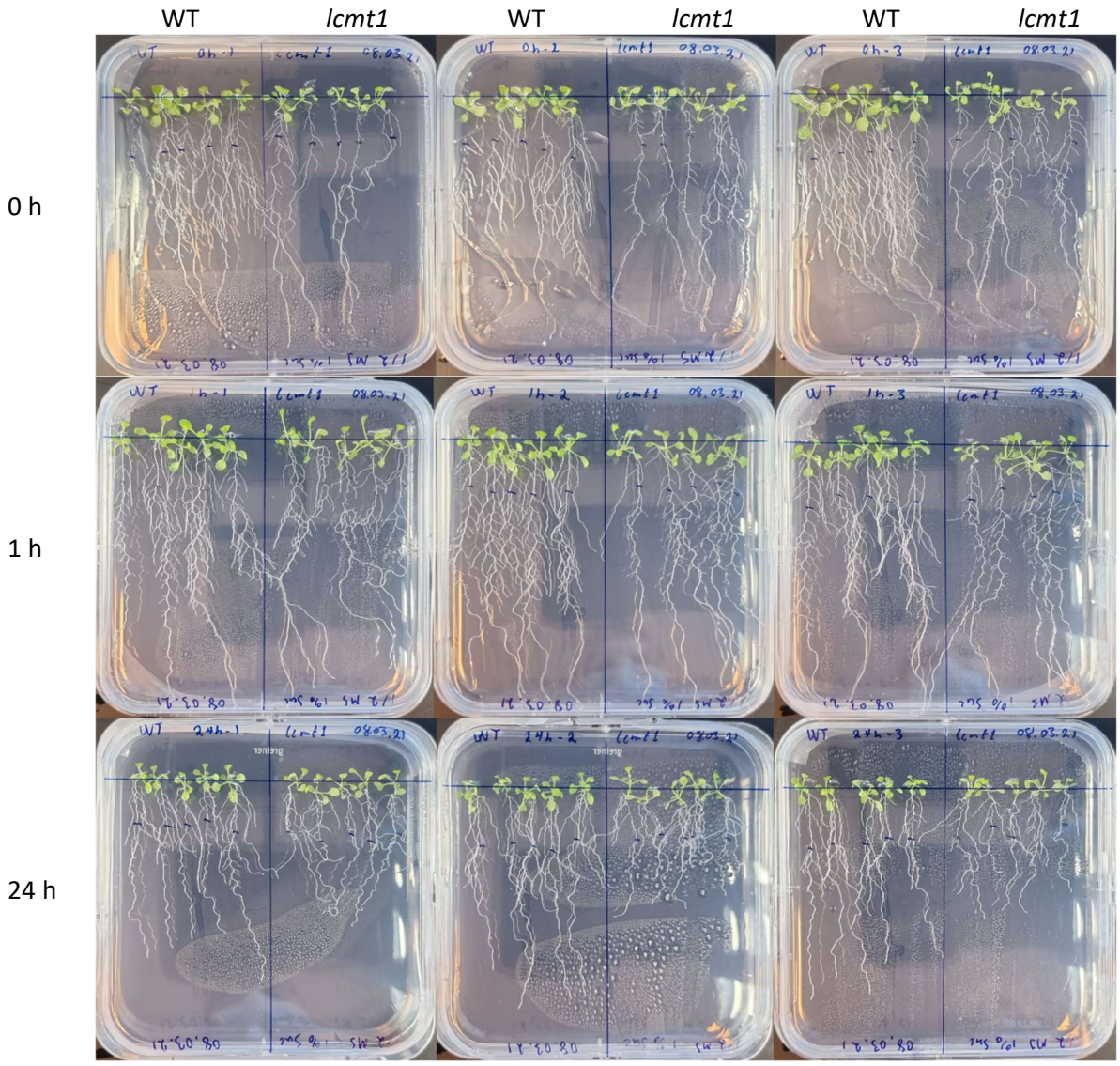


Figure 3.5.3: Third parallel of heat treatment recovery experiment 1. The growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. The root length was marked immediately before start of heat treatment.

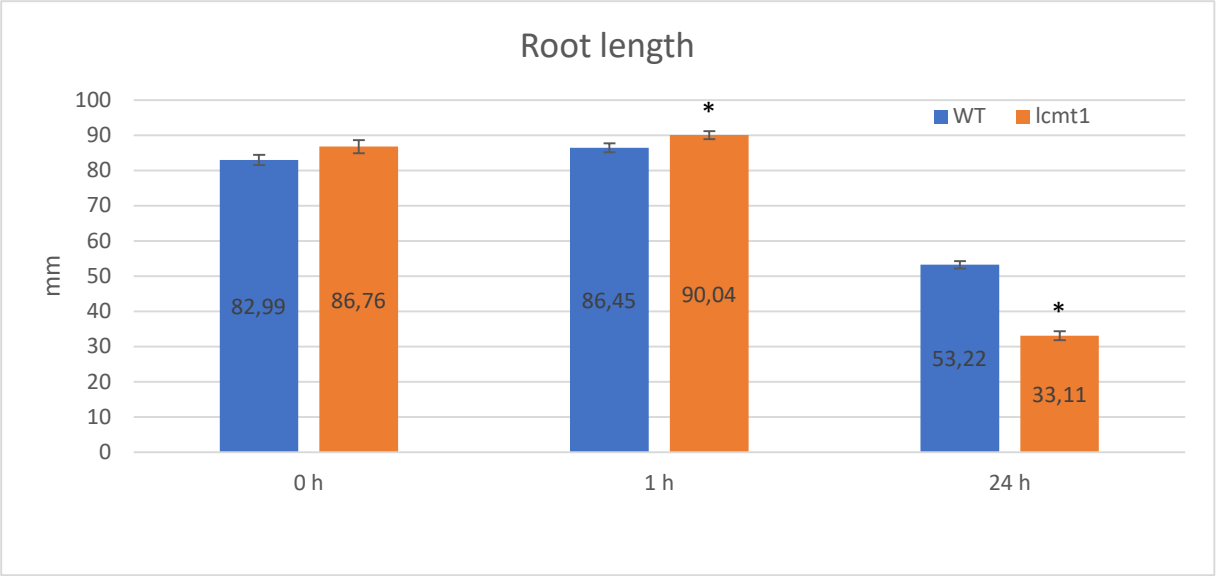


Figure 3.5.4: The mean root length growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. WT showed shorter growth than *lcmt1* in the two first groups, but *lcmt1* showed more erratic growth. After 24 h *lcmt1* showed significantly reduced growth compared to WT. n=45, SE is shown as vertical bars. The values for 1 h and 24 h were significant (star), $p<0.05$.

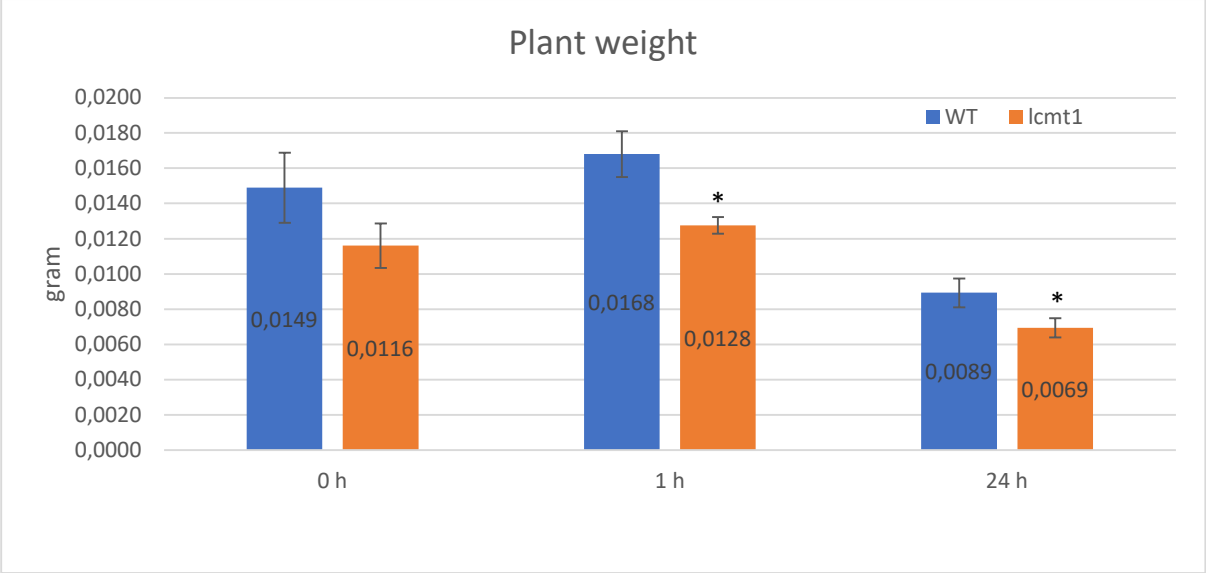


Figure 3.5.5: The mean plant weight of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. WT showed increased growth compared to *lcmt1* in all groups. Both WT and *lcmt1* indicated positive growth after 1 h heat treatment compared to the control. After 24 h *lcmt1* showed significantly reduced growth compared to WT. n=9, SE is shown as vertical bars. Values for 1h and 24h were significant (star), $p<0.05$.

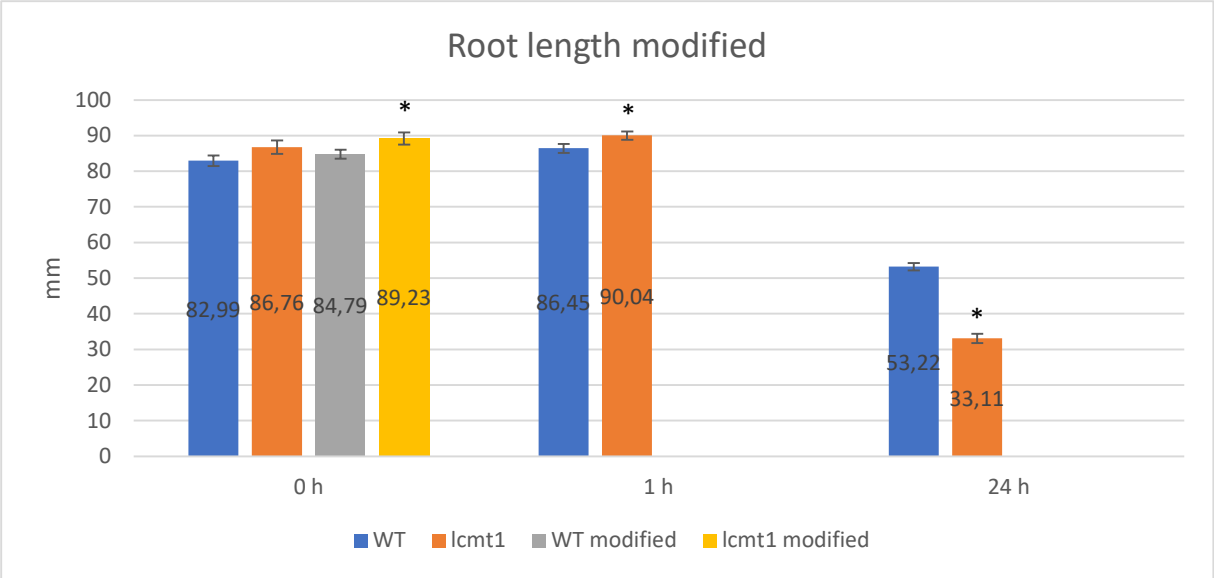


Figure 3.5.6: The mean root length growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. WT showed shorter growth than *lcmt1* in the two first groups, but *lcmt1* showed more erratic growth. After 24 h *lcmt1* showed significantly reduced growth compared to WT. Included results with the omitted anomaly. n=45, n=40 for 0 h without the anomaly plate. SE is shown as vertical bars. All values but the unmodified control were significant (star), $p<0.05$.

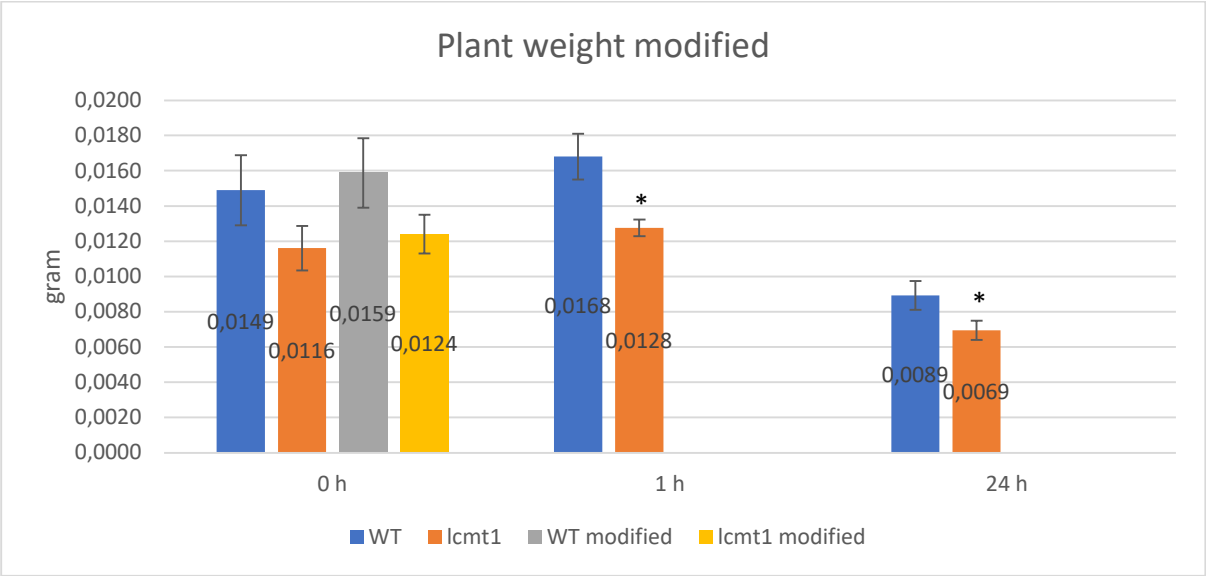


Figure 3.5.7: The mean plant weight of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. WT showed increased growth compared to *lcmt1* in all groups, and significantly so for the 1 h and 24 h groups. Both WT and *lcmt1* showed positive growth after 1 h heat treatment compared to the control. n=9, n=8 for 0 h without the anomaly plate. SE is shown as vertical bars. Values for 1 h and 24 h were significant (star), $p<0.05$.

3.6 ½ MS heat treatment recovery experiment 2: Root length and shoot weight after one week

The second full ½ MS heat treatment experiment showed in figure 3.6.4 and 3.6.5 WT outperforming *lcmt1* in every group in both root length and shoot weight with significant values, $p<0.05$. This experiment had three parallels and was repeated three times like the previous experiment. The standard errors were much tighter on shoot weight compared to plant weight in the previous experiment which showed that shoot weight was a better value to monitor. Interestingly WT root length were longer than *lcmt1* in the control and 1 h group in this experiment in contrast with the other two experiments. Figure 3.6.1 – 3.6.3 showed more branching of roots and longer branches of roots in WT compared to *lcmt1* reaffirming this observation from previous experiments. WT showed more consistent root lengths while *lcmt1* showed more consistent shoot weight, although WT were reliably heavier (figures 3.6.4 and 3.6.5). The mutant *lcmt1* showed more erratic growth and in the 24 h group even splitting of the root growth resulting in two main roots of much shorter length in some cases. In the 24 h group *lcmt1* showed some yellowing of leaves suggesting chlorosis and even one case of withering.

Figures 3.6.4 and 3.6.5 showed WT performed significantly better than *lcmt1* in all groups for both root growth and shoot weight.

It should be noted that the same heat chamber with the same settings for 37°C were used for every experiment, but in this experiment the samples were kept about 20 cm higher than in the other experiments. This could have exposed the plants to a slightly higher temperature and may explain the difference in results between this experiment and the previous one. This may indicate there to be a threshold value at 37°C, especially for the 24 h group with several examples of miniscule root length growth for *lcmt1* (figure 7.4.1).

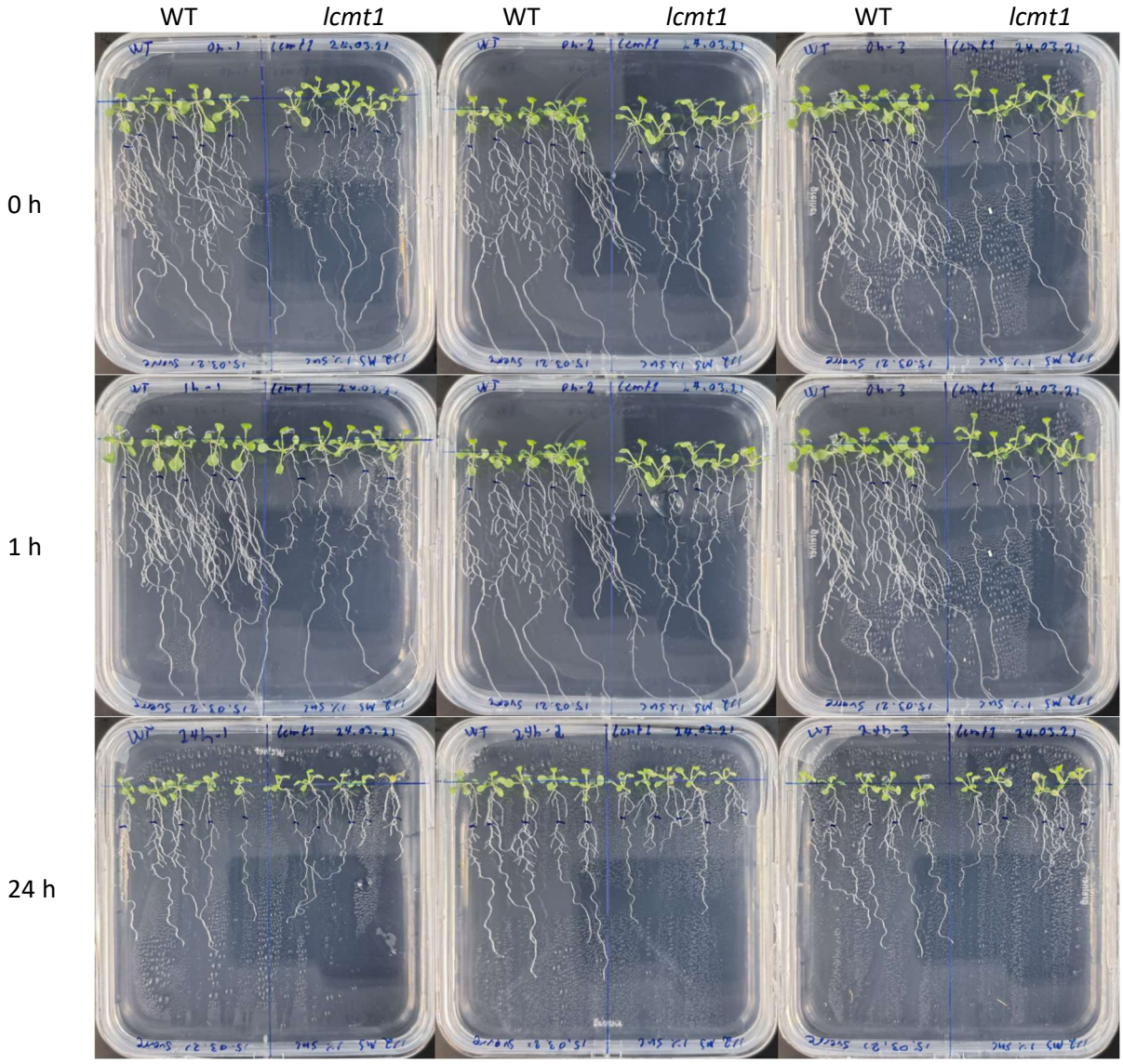


Figure 3.6.1: First parallel of heat treatment recovery experiment 2. The growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. The root length was marked immediately before start of heat treatment.

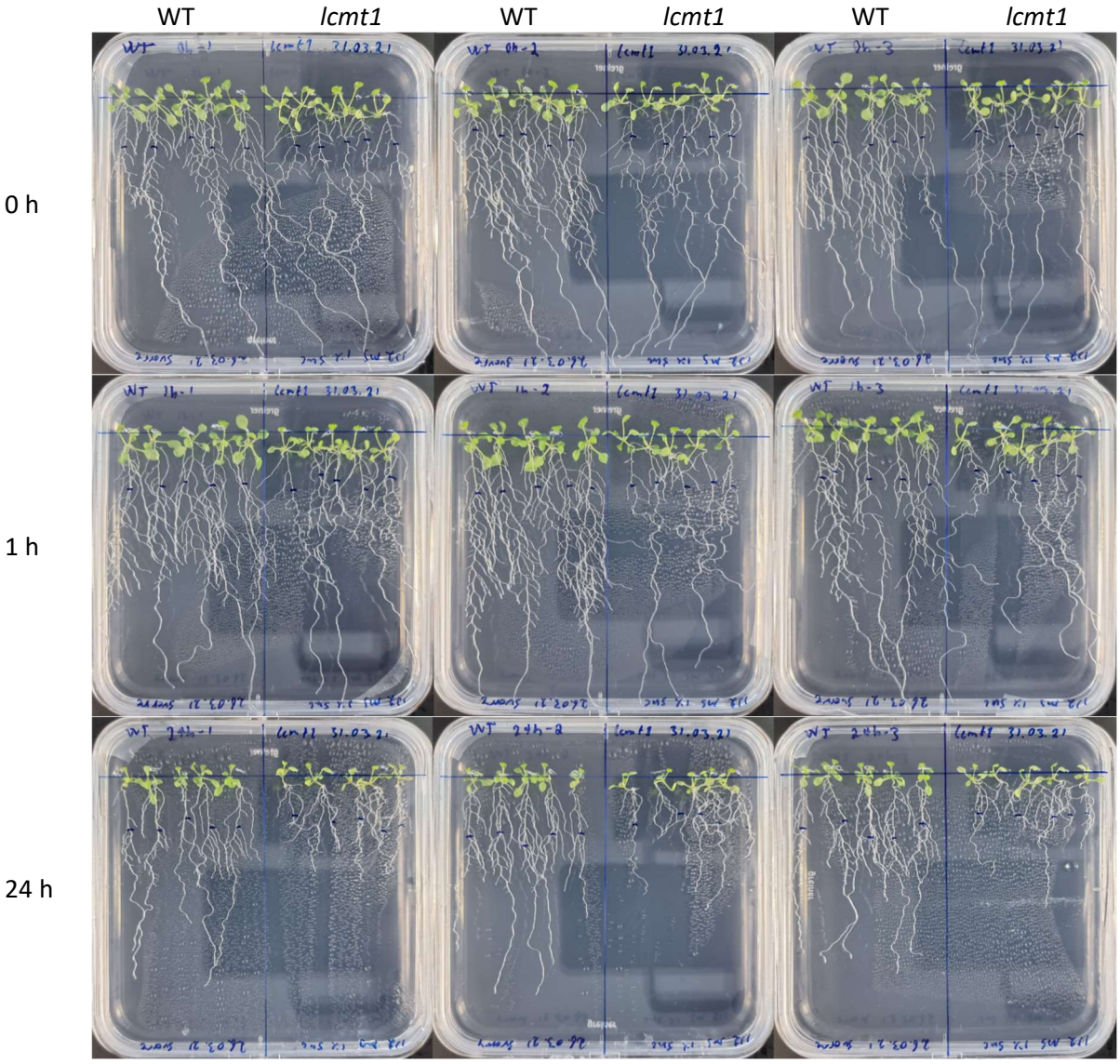


Figure 3.6.2: Second parallel of heat treatment recovery experiment 2. The growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. The root length was marked immediately before start of heat treatment.

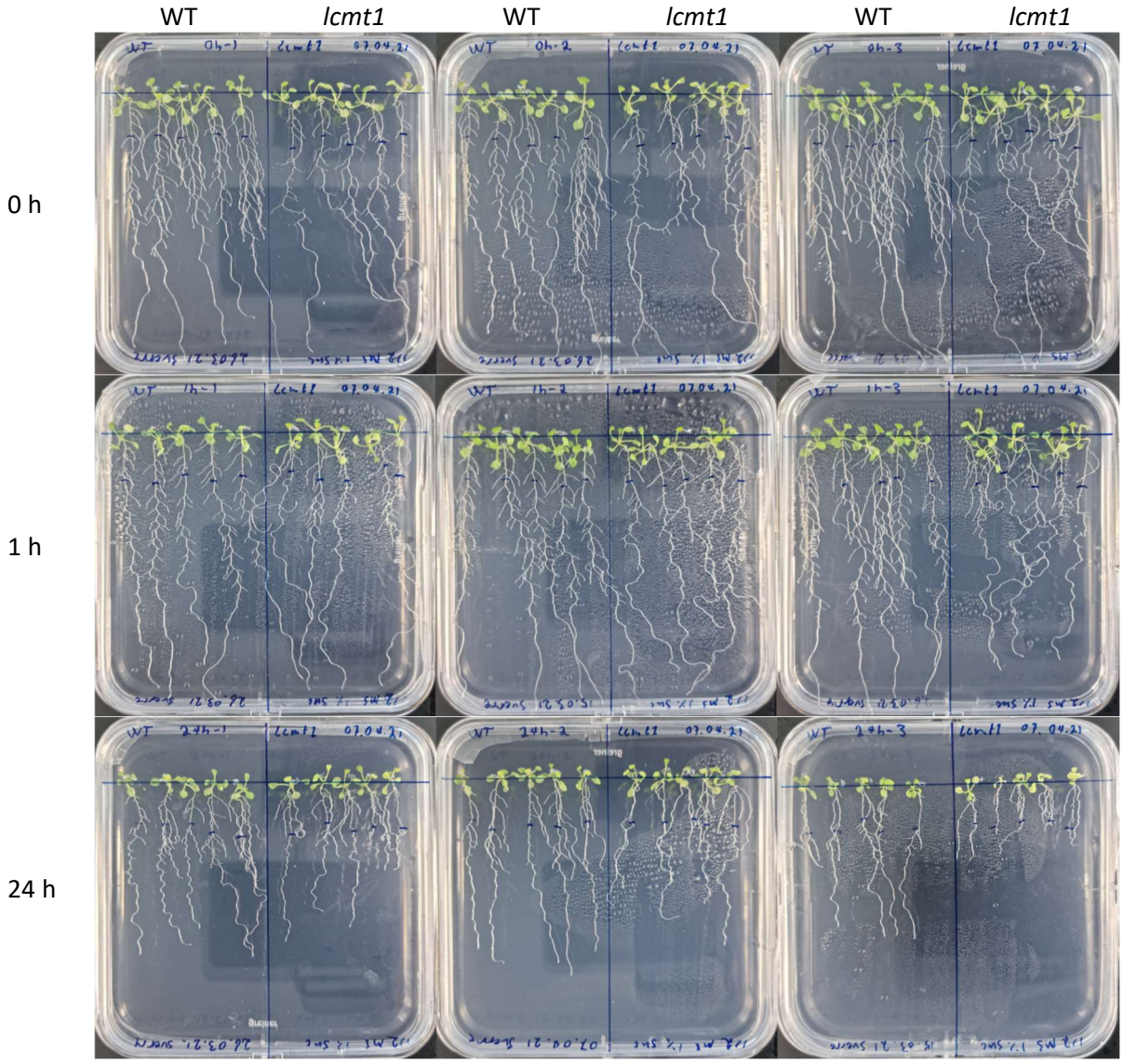


Figure 3.6.3: Third parallel of heat treatment recovery experiment 2. The growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. The root length was marked immediately before start of heat treatment.

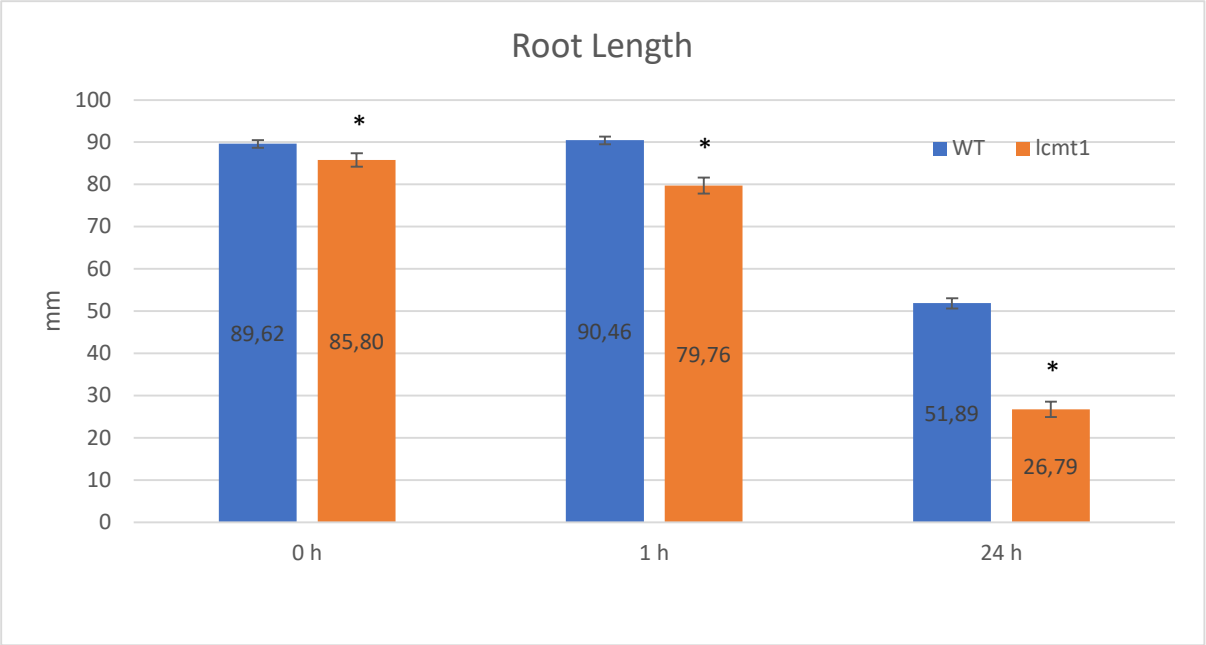


Figure 3.6.4: The mean root length growth of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. WT showed significantly longer growth than *lcmt1* in all groups. There were little observable difference after 1 h heat treatment, if any the effects on WT were slightly positive and on *lcmt1* slightly more but negative. After 24 h both WT and *lcmt1* showed strongly reduced growth and *lcmt1* significantly more than WT. n=45, SE is shown as vertical bars. All values were significant (star), $p<0.05$.

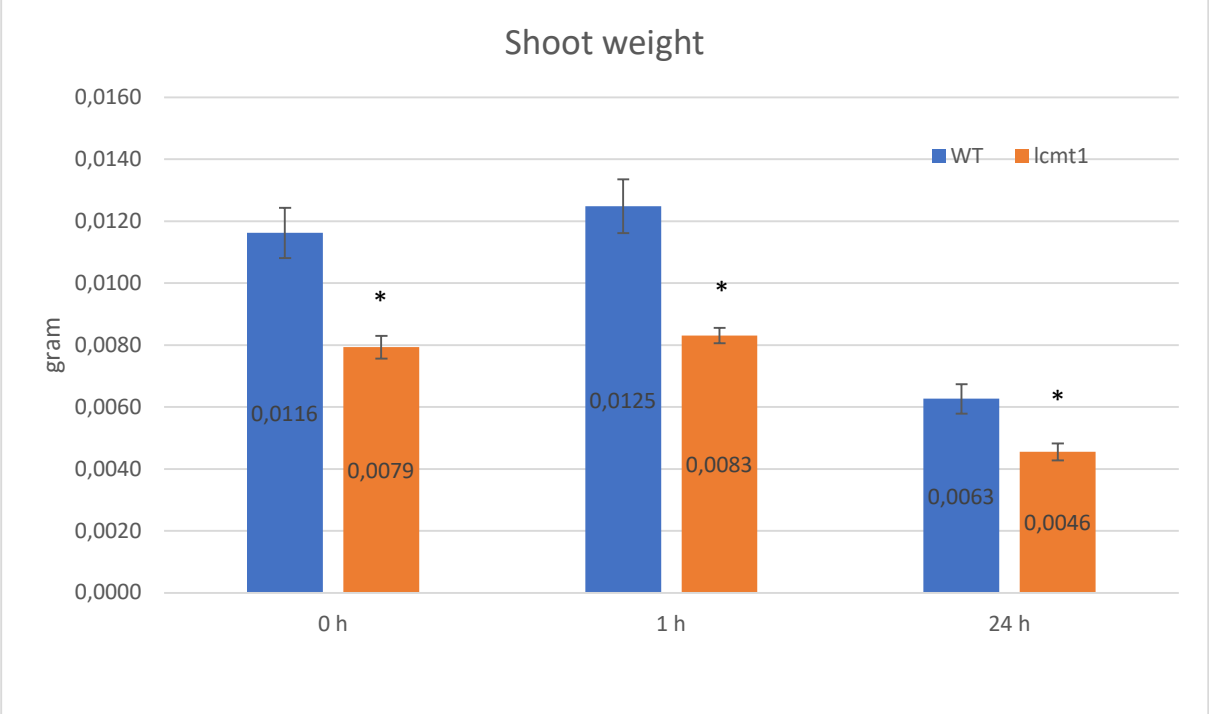


Figure 3.6.5: The mean shoot weight of *Arabidopsis thaliana* WT and *lcmt1* on ½ MS media seven days after the start of heat treatment at 37°C for 0, 1 and 24 h. For the remainder of the time the plants were kept in an artificial 16 h light/8 h dark cycle at approximately 22°C. WT showed significantly increased growth compared to *lcmt1* in all groups. Both WT and *lcmt1* showed positive growth after 1 h heat treatment compared to the control. After 24 hours both WT and *lcmt1* showed strongly reduced growth and *lcmt1* significantly more than WT. n=9, SE is shown as vertical bars. All values were significant (star), $p<0.05$.

3.7 RNA concentration

The seedlings were grown in Petri dishes as described in section 2.5. Seeds were planted and put in a dark and cold place. After two days they were moved to a growth room with an artificial 16 h light/8 h dark cycle with a temperature of 22°C. After an additional five days the seedings were exposed to the different levels of heat treatment and they were then ground up with a pestle and mortar using liquid nitrogen. While deep frozen the different dry samples were weighed up to 100 mg as accurately as possible, but not over. The two extractions originated from two different samples of seedlings. The extraction process is listed in detail in section 2.6. With the extracted samples, the RNA was analysed using Thermo Fischer's Nanodrop One spectrophotometer. After cleaning the lens, a blank was used to calibrate the machine. All the samples were scanned, and the lens was wiped in between each scan. The results from both extractions are listed in tables 3.7.1 and 3.7.2. The ratio value 260/280 indicate purity of RNA and should be over 2.0 to be considered pure. A lower value may indicate contaminants like proteins absorbing in the 280 nm range. The ratio value 260/230 also measures purity and should also be above 2.0. A lower value may indicate contaminants of other molecules like phenols or carbohydrates absorbing in the 230 nm range (Scientific, 2007).

Table 3.7.1: Nanodrop values from the first RNA extraction. RNA was extracted from about 200 whole seedlings after five days of growth and the different levels of heat treatment. All values for both 280 nm and 230 nm were considered pure.

	Concentration (ng/µL)	Ratio (A260/A280)	Ratio (A260/A230)
WT-0h	763.1	2.13	2.29
***lcmt1*-0h**	949.7	2.12	2.44
WT-1h	1142.0	2.14	2.45
***lcmt1*-1h**	852.9	2.13	2.32
WT-24h	555.2	2.10	2.43
lcmt1-24h	481.5	2.13	2.42

Table 3.7.2: Nanodrop values from the second RNA extraction. RNA was extracted from about 100 whole seedlings after five days of growth and the different levels of heat treatment. All values for both 280 nm and 230 nm were considered pure.

	Concentration (ng/µL)	Ratio (A260/A280)	Ratio (A260/A230)
WT-0h	1793.0	2.16	2.48
***lcmt1*-0h**	1070.7	2.15	2.46
WT-1h	1366.7	2.16	2.44
***lcmt1*-1h**	997.6	2.15	2.45
WT-24h	1392.9	2.14	2.49
lcmt1-24h	593.7	2.13	2.39

All values from the nanodrop scans were considered pure, although some concentrations were a bit low. However, the concentrations were all more than high enough to perform the necessary RT-PCR gene expression tests.

3.8 Gene expression experiment

Figures 3.8.1 and 3.8.2 showed little variation in the C_Q values of UBC35 in the different samples in both runs. This indicated that it was a good endogenous control. Low values meaning few cycles before detecting a signal indicated high levels of RNA. The lowest values were seen for HSP90-1 in the groups exposed to heat treatment, while the highest values were seen in IRT1 in the 24 h groups. These results were reflected in figures 3.8.3 and 3.8.4 where both runs showed the RQ values extreme elevation of HSP90-1 for both WT and *lcmt1* exposed to both 1 h and 24 h of heat, although the values were highest in the 1 h group. Both runs also showed an extreme decrease in RQ values in IRT1 for both WT and *lcmt1* in the 24 h group. The values were lower in the 1 h groups compared to the control as well, but compared to the 24 h group, only marginally so.

Figures 3.8.5 and 3.8.6 showed the RQ values within HSP90-1 and IRT1 respectively easily comparing them between WT and *lcmt1*. Figure 3.8.6 showed a decrease with elevated intervals of heat treatment, but also elevated levels within the mutant *lcmt1* compared to WT in all groups. Figure 3.8.5 clearly showed the extreme elevation of RQ values in all groups exposed to heat treatment compared to the control, but the only trend when comparing WT with *lcmt1* was a decreased value in the mutant *lcmt1* in the 24 h group compared with WT.

Figure 3.8.7 showed the RQ values of APXS. Although the values were far from as extreme as with HSP90-1 and IRT1 there were still differences between the groups. The two runs showed consistent decreased values in the 1 h group and consistent elevated values in the 24 h group. However, the only consistency between the two runs when comparing WT with *lcmt1* were decreased values in the mutant *lcmt1* in the 24 h group compared with WT. The extreme value of WT in the 24 h group in the first run was not replicated in the second run. That extreme value should maybe be disregarded, but ideally a third run should be conducted to verify those results.

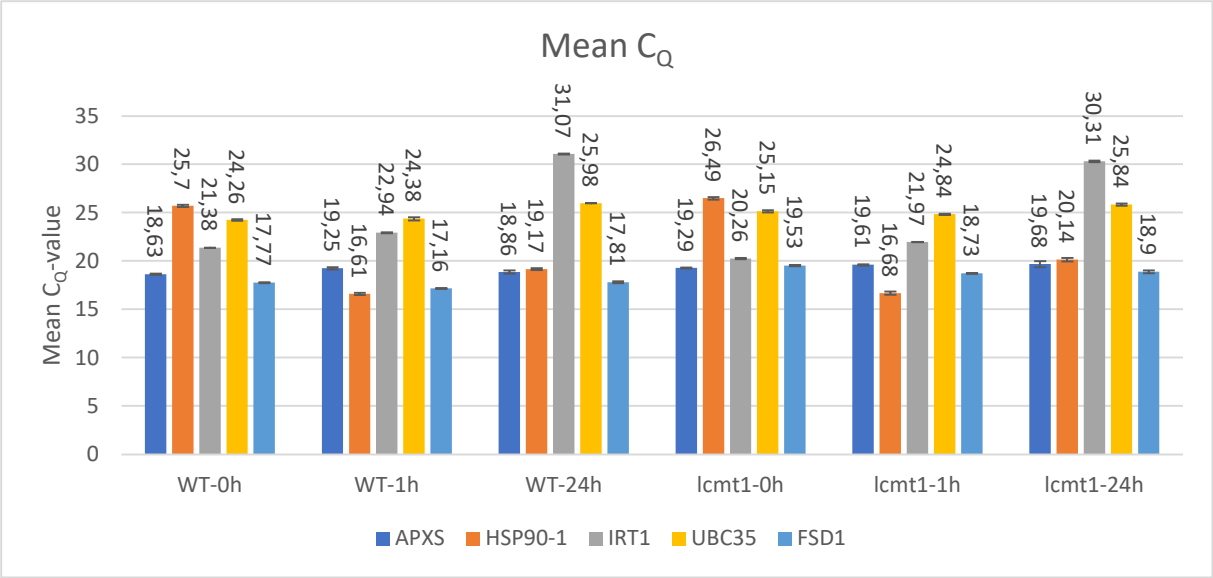


Figure 3.8.1: First gene expression run results. Mean C_Q values for the genes APXS, FSD1, HSP90-1 and IRT1 expressed in about 200 whole seedlings of *Arabidopsis thaliana* WT and *lcmt1*. The plants were exposed to 0, 1 and 24 h heat treatment each. UBC35 was used as the endogenous control. SE is shown as vertical bars.

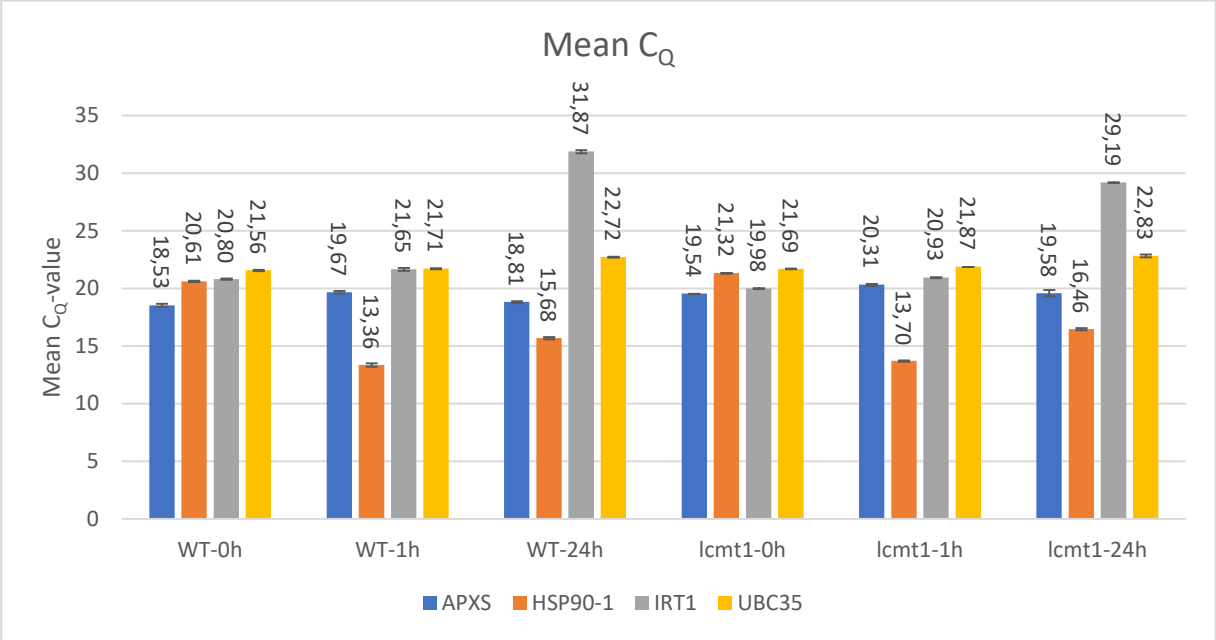
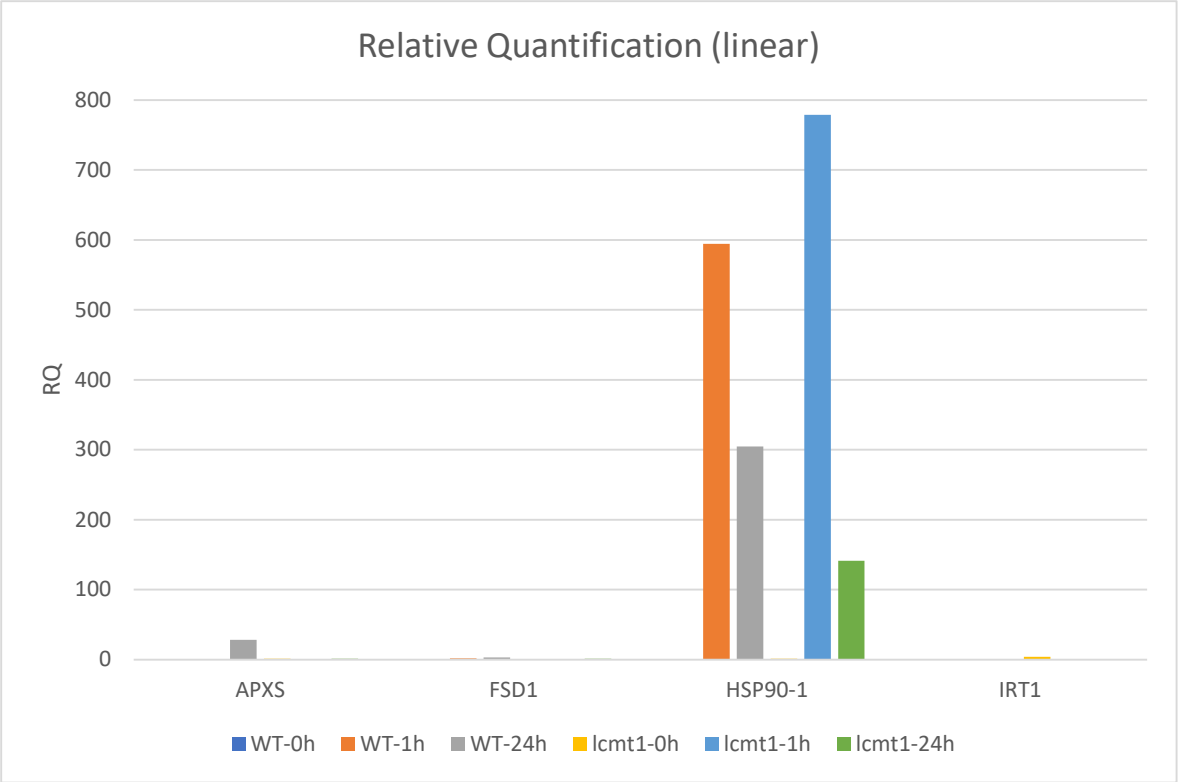
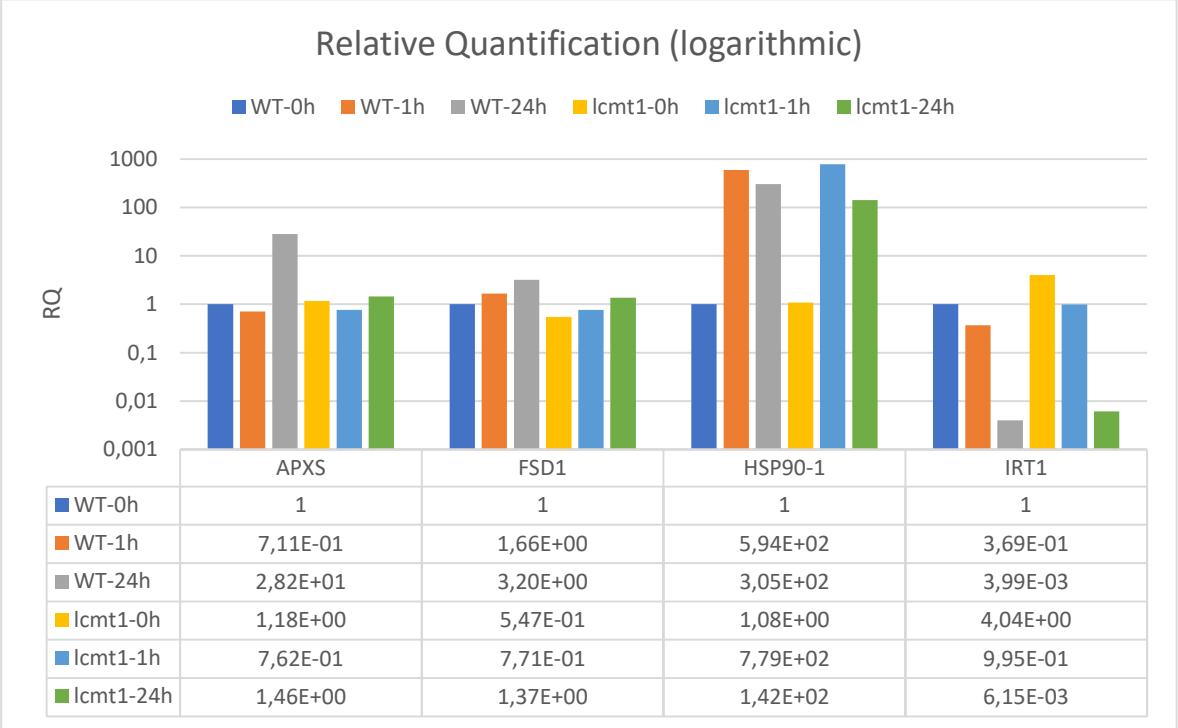


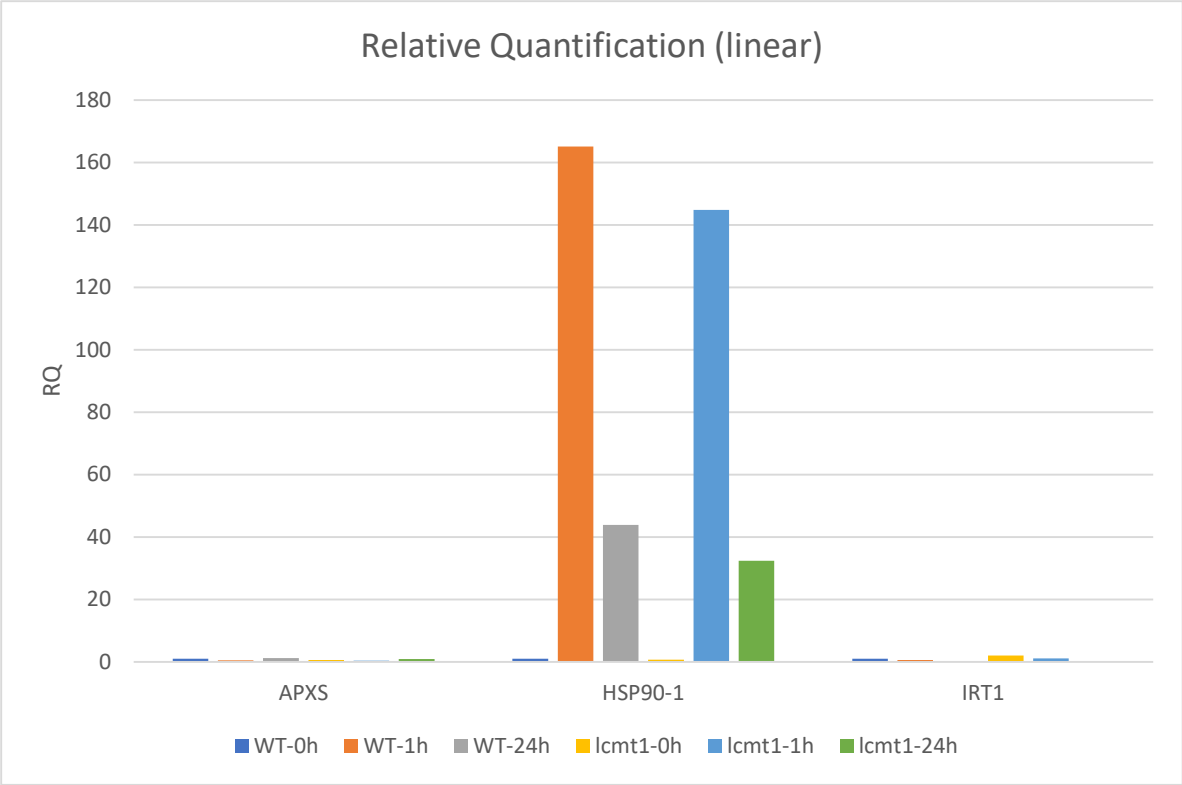
Figure 3.8.2: Second gene expression run results. Mean C_Q values for the genes APXS, HSP90-1 and IRT1 expressed in about 100 whole seedlings of *Arabidopsis thaliana* WT and lcmt1. The plants were exposed to 0, 1 and 24 h heat treatment each. UBC35 was used as the endogenous control. SE is shown as vertical bars.

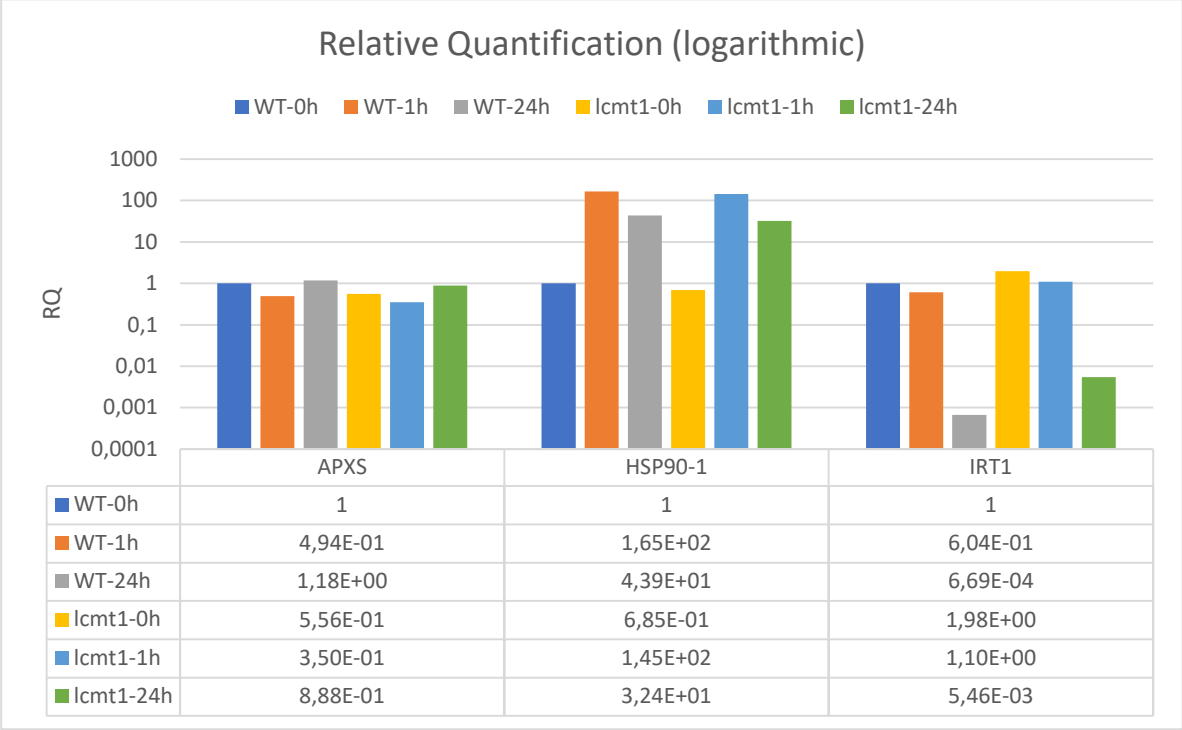




	APXS	FSD1	HSP90-1	IRT1
WT-0h	1	1	1	1
WT-1h	7,11E-01	1,66E+00	5,94E+02	3,69E-01
WT-24h	2,82E+01	3,20E+00	3,05E+02	3,99E-03
lcmt1-0h	1,18E+00	5,47E-01	1,08E+00	4,04E+00
lcmt1-1h	7,62E-01	7,71E-01	7,79E+02	9,95E-01
lcmt1-24h	1,46E+00	1,37E+00	1,42E+02	6,15E-03

Figure 3.8.3: Mean RQ values from the first gene expression run. Mean RQ values for the genes APXS, FSD1, HSP90-1 and IRT1 expressed in about 200 whole seedlings of *Arabidopsis thaliana* WT and *lcmt1*. The plants were exposed to 0 h, 1 h and 24 h heat treatment each. UBC35 was used as the endogenous control and WT-0h was used as the run calibrator. The graph is rendered both linearly and logarithmically because a linear graph is more conventional, but in this case a logarithmic one was much more informative. The HSP90-1 values were extremely elevated when exposed to the heat treatment and the values for IRT1 were extremely decreased when exposed to 24 hours of heat compared to all other values. A logarithmic scale was needed because values within a single gene spanned over 1000 points.





	APXS	HSP90-1	IRT1
WT-0h	1	1	1
WT-1h	4,94E-01	1,65E+02	6,04E-01
WT-24h	1,18E+00	4,39E+01	6,69E-04
lcmt1-0h	5,56E-01	6,85E-01	1,98E+00
lcmt1-1h	3,50E-01	1,45E+02	1,10E+00
lcmt1-24h	8,88E-01	3,24E+01	5,46E-03

Figure 3.8.4: Mean RQ values from the second gene expression run. Mean RQ values for the genes APXS, HSP90-1 and IRT1 expressed in about 100 whole seedlings of *Arabidopsis thaliana* WT and *lcmt1*. The plants were exposed to 0 h, 1 h and 24 h heat treatment each. UBC35 was used as the endogenous control and WT-0h was used as the run calibrator. The graph is rendered both linearly and logarithmically because a linear graph is more conventional, but in this case a logarithmic one was much more informative. The HSP90-1 values were extremely elevated when exposed to the heat treatment and the values for IRT1 were extremely decreased when exposed to 24 h of heat compared to all other values. A logarithmic scale was needed because values within a single gene spanned over 1000 points.

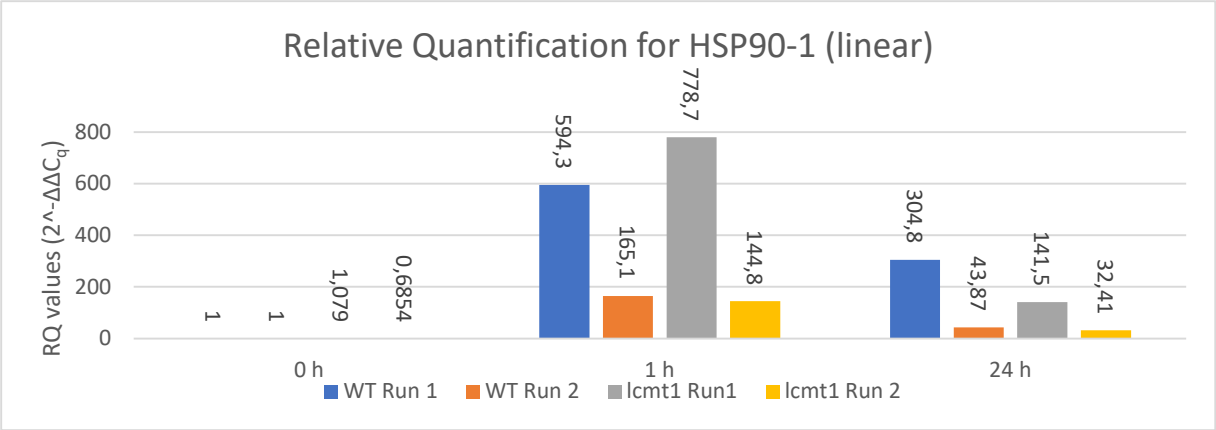


Figure 3.8.5: Comparing the RQ numbers for HSP90-1 between the two runs. Even though the values differed greatly between the two runs, the trend was the same. There was an extremely elevated value for both 1 h and 24 h, but the value was highest in the 1 h group. This was true for both WT and *lcmt1*. Values in the 0 h group were too small to be seen. A conventional linear scale figure was used here, see figure 7.5.1 for a logarithmic one.

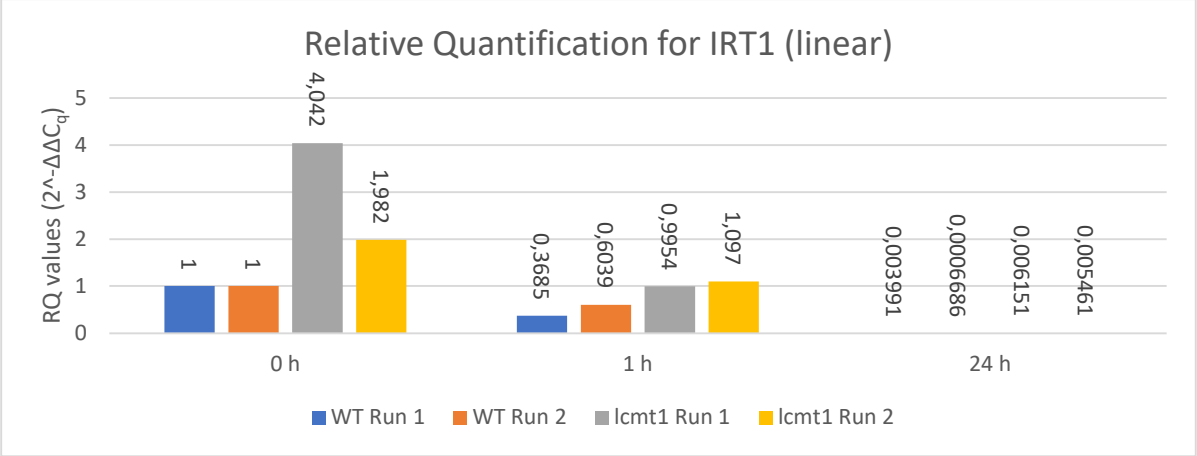


Figure 3.8.6: Comparing the RQ values for IRT1 between the two runs. Even though the values differ greatly between the two runs, the trend was the same. There was an extremely decreased value in the 24 h group. This was true for both WT and *lcmt1*, but even more so for WT. Values in the 24 h group were too small to be seen. A conventional linear scale figure was used here, see figure 7.5.2 for a logarithmic one.

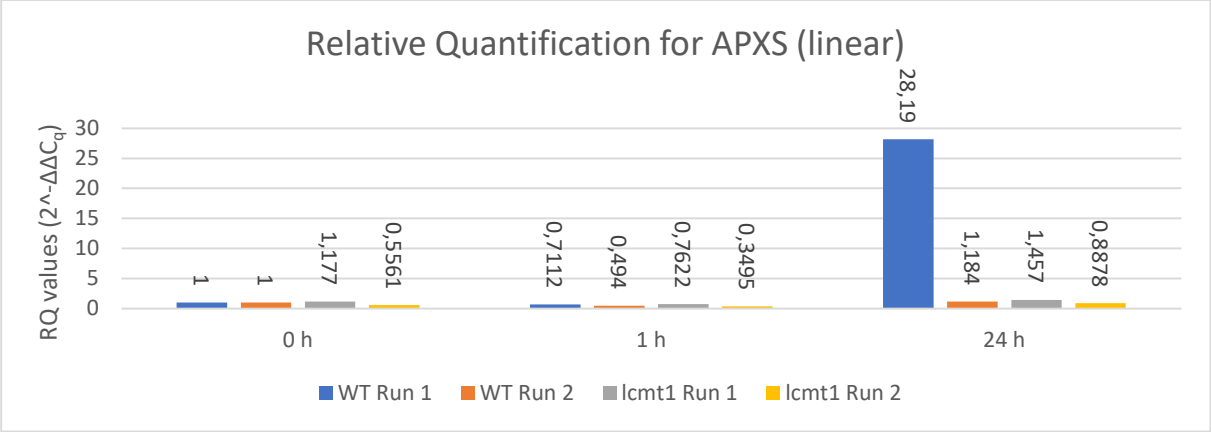


Figure 3.8.7: Comparing the RQ values for APXS between the two runs. They showed the same trend and ignoring the extreme value for WT in the 24 h group, the values were much less extreme than HSP90-1 and IRT1. Both WT and *lcmt1* showed a decreased value in the 1 h group, but an elevated value in the 24 h group compared to the control. A conventional linear scale figure was used here, see figure 7.5.3 for a logarithmic one.

4. Discussion

4.1 Phenotype of *Arabidopsis thaliana* grown in soil

Observations of the phenotype of *A. thaliana* grown in soil indicated WT growing broader thicker leaves with deeper green colour, indicating faster growth and an overall healthier plant compared with *lcmt1*. In the control groups the mutant *lcmt1* was consequently observed producing the central stem earliest with blooming flowers. WT on the other hand took longer to do the same with any stems being shorter and the majority of the plants not even having started shooting of the stem. With increased heat stress WT and *lcmt1* displayed opposite behaviour, WT having an increase in blooming flowers while *lcmt1* having a decrease in blooming flowers. This seemed to coincide with section 1.4 that mention annual plants having an increased tendency to reproduce with increased stress (Taiz, et al., 2015), at least regarding WT. The mutant *lcmt1* on the other hand may have already been stressed due to its mutant defect, and its decrease in blooming flowers may have been it being past its ability to cope with the extra stress.

4.2 Phenotype, root length and shoot weight of *Arabidopsis thaliana* grown in Petri dishes

In general the observations of the phenotype of *A. thaliana* in Petri dishes indicated WT producing fuller roots with more branching and more elongation of the branching roots compared with *lcmt1*. Measurements of the root lengths on the other hand did not show a clear difference between the two, at least not in the control group. However, after 24 h of heat stress WT showed consequently significant longer root growth compared with *lcmt1*.

Values for plant weight from section 3.4 indicated WT outperforming *lcmt1*, but only with significance under stress. This may be due to some difficulty to get good readings on plant weight. However, values for shoot weight from section 3.5 were consequently and with significance in favour of WT in every group.

4.3 Gene expression analysis

The two runs both showed the same trends in gene expression, although with great variation in values. They both showed an extreme increase in the RQ values for HSP90-1 after both 1 h and 24 h of heat treatment, and an extreme decrease in RQ values for IRT1 in the 24 h group. The most consistently noticeable difference between WT and *lcmt1* was in IRT1 where *lcmt1* had the highest value every time. It can also be mentioned that WT had the highest values of both HSP90-1 and APXS in the 24 h group in both runs.

5. Conclusion and future perspective

5.1 Conclusion

All results point to the mutant *lcmt1* with a low methylation level of PP2A has a lower threshold for maintaining normal growth under heat stress. It even showed signs of stress under normal conditions. Values for shoot weight showed it underperformed compared with WT in every group. Values for root length were not especially affected under normal conditions or under short periods of heat stress although some values were significant. However, *lcmt1* severely underperformed compared with WT in the 24 h group. WT also displayed low values of root growth in the 24 h group, but there were several instances of *lcmt1* hardly growing at all.

Regarding the gene expression results the mutant *lcmt1* displayed the highest values of IRT1 in every group in both runs. However, WT displayed the strongest response in IRT1 values which were decreased under heat stress. Regarding HSP90-1 and APXS WT had the highest values for both in the 24 h group in both runs. Values for HSP90-1 were extremely elevated under heat stress, with the strongest response in the 1 h group. Values for APXS were fairly consistent, but somewhat decreased in the 1 h group and somewhat elevated in the 24 h group.

5.2 Future perspective

Results showed the mutant *lcmt1* performed worse compared with WT in recovery experiment 2 in Petri dished than in recovery experiment 1. This may be because the temperature was slightly higher in recovery experiment 2. It would be interesting to study this further to see if a threshold point can be found for *lcmt1* and see how far this would be from WT.

There were also some results indicating shorter periods of heat beneficial to the plants. Increased enzyme activity is suspected. There may be a difference in performance between WT and *lcmt1* there, but it may have very narrow limits and prove difficult to explore.

6. References and web pages

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7. Appendix

7.1 Soil experiment

Table 7.1.1: Number of flowering plants at end of experiment.

		0 h	8 h	18 h	24 h
Parallel 1	WT	6	10	10	0
	lcmt1	13	11	4	0
Parallel 2	WT	2	0	5	0
	lcmt1	15	15	8	0
Parallel 3	WT	4	4	3	0
	lcmt1	15	15	3	0

7.2 Trial ½ MS heat treatment experiment

Table 7.2.1:Trial ½ MS heat treatment experiment root lengths measured in mm.

	WT-0h	lcmt1-0h	WT-24h	lcmt1-24h
Plate 1	77,75	75,07	37,53	13,2
	73,58	78,85	37,7	19,49
	74,46	82,85	12,65	9,65
	77,45	74,05	38,11	7,9
	75,14	77,55	39,9	23,79
Plate 2	76,07	80,61	25,64	4,94
	78,27	79,38	45,48	4,16
	73,58	74,62	42,41	12,24
	78,17	75,58	48,09	15,03
	66,19	79,86	38,97	18,79
Plate 3	73,15	68,42	26,02	16,29
	71,55	73,33	24	16,16
	65,94	72,07	6,41	12,39
	60,21	68,98	27,1	21,44
		72,94	24,12	27,6
mean	72,965	75,61067	31,60867	14,87133
SD	5,375066	4,210296	11,993	6,709245
SE	1,436547	1,087094	3,096579	1,73232
ttest	0,075062666		3,00462E-05	

Table 7.2.2: ½ MS heat treatment recovery experiment 1 average shoot weight measured in gram.

	WT-0h	lcmt1-0h	WT-24h	lcmt1-24h
Plate 1	0,0088	0,0061	0,00262	0,00236
Plate 2	0,0066	0,0056	0,00464	0,00336
Plate 3	0,0051	0,0042	0,00248	0,00294
Mean	0,0068333	0,0053	0,0032467	0,002887
SD	0,001861	0,0009849	0,0012087	0,000502
SE	0,0010744	0,0005686	0,0006978	0,00029
ttest	0,137866997		0,329321133	

7.3 ½ MS heat treatment experiment 1

Table 7.3.1: ½ MS heat treatment recovery experiment 1 root lengths measured in mm.

		WT-0h	*lcmt1*-0h	WT-1h	*lcmt1*-1h	WT-24h	*lcmt1*-24h
Parallel 1	Plate 1	86,45	95,87	83,51	82,21	57,12	38,28
		85,71	98,17	89,16	95,41	48,95	31,19
		99,54	95,37	91,24	90,65	55,1	35,07
		85,77	90,49	82,43	89,5	56,41	33,43
		92,03	79,58	84,62	87,1	54,63	29,84
	Plate 2	58,71	75,69	85,4	92,77	47,57	45,65
		70,53	77,98	87,48	100,61	40,82	39,11
		57,46	64,49	94,41	93,83	49,69	37,98
		67,43	64,48	90,7	84,56	48,41	31,28
		88,82	52,12	86,4	90,85	52,52	29,82
	Plate 3	87,5	104,79	87,27	100,24	43,16	28,57
		94,32	104,11	88,69	86,51	50,63	22,59
		88,91	97,79	93,6	91,23	43,89	49,76
		88,57	103,08	87	99,84	45,5	27,05
		92,4	98,74	72,93	90,5	50,87	44,49
Parallel 2	Plate 1	69,37	95,51	91,75	90,92	58,03	43,5
		80,14	89,31	87,4	97	45,6	46,11
		76,24	66,99	90,27	89,53	55,24	13,73
		65,38	76,41	88,25	91,49	56,88	15,03
		78,33	60,52	99,91	61,33	54,93	10,37
	Plate 2	84,69	99,91	45,37	92,79	48,9	47,32
		88,38	89,21	71,84	92,37	51,41	39,41
		79,79	105,52	85,48	97,6	62,66	35,36
		63,7	75,79	77,96	89,48	32,98	38,03
		92,7	97,81	95,22	88,08	52,58	29,11
	Plate 3	78,77	92,34	76,17	95,24	56,48	27,2
		85,96	92,51	85,66	98	59,78	27,71
		94,13	97,44	95,23	99,53	54,21	38,24
		69,95	95,79	88,18	87,06	62,49	28,43
		80,93	93,07	90,64	80,65	61,58	22,81
Parallel 3	Plate 1	85,19	94,42	82,01	85,77	54,91	33,3
		90,4	85,76	87,85	90,52	49,76	45,15
		88,43	87,55	90,69	91,29	46,15	41,24
		90,45	77,49	90,3	102,62	49,14	35,63
		82,79	81,54	95,28	70,79	64,19	36,42
	Plate 2	82,11	85,02	84,33	91,69	49,27	34,73
		85,57	91,5	91,7	87,04	54,49	31,28
		77,79	75,34	81,05	95,21	67,01	31,89
		79,46	73,98	86,61	88,21	59,03	18,91
		88,52	79,28	86,17	89,71	65,52	26,93
	Plate 3	84,58	97,34	91,17	88,1	60,32	36,29

		93,68	84,55	87,09	94,04	54,45	35,77
		92,14	95,25	88,22	87,89	61,25	30,53
		87,2	83,6	89,33	88,52	51,38	31,43
		93,57	80,56	84,5	73,33	49,22	33,89
	mean	82,98867	86,75689	86,45489	90,03578	53,22467	33,108
	SD	9,904258	12,64086	8,388988	7,690284	6,89893	8,733045
	SE	1,47644	1,884388	1,250557	1,1464	1,028432	1,301846
	ttest	0,059527759		0,01881558		9,01023E-21	

Table 7.3.2: ½ MS heat treatment recovery experiment 1 average plant weight measured in gram.

		WT-0h	lcmt1-0h	WT-1h	lcmt1-1h	WT-24h	lcmt1-24h
Parallel 1	Plate 1	0,01802	0,01316	0,0184	0,01148	0,0068	0,00548
	Plate 2	0,00704	0,0052	0,01636	0,0139	0,00722	0,00502
	Plate 3	0,013	0,0132	0,01922	0,01256	0,00606	0,00524
Parallel 2	Plate 1	0,00832	0,00996	0,013	0,01286	0,00826	0,00572
	Plate 2	0,01256	0,01846	0,0112	0,01032	0,00772	0,00846
	Plate 3	0,01096	0,0119	0,01192	0,01152	0,00972	0,008
Parallel 3	Plate 1	0,0194	0,0078	0,021	0,014	0,0106	0,0092
	Plate 2	0,0198	0,0136	0,0186	0,0146	0,014	0,0086
	Plate 3	0,025	0,0112	0,0216	0,0136	0,01	0,0068
	Mean	0,0149	0,0116089	0,0168111	0,01276	0,008931	0,006947
	SD	0,0059759	0,0037814	0,0039074	0,001419	0,002446	0,00164
	SE	0,001992	0,0012605	0,0013025	0,000473	0,000815	0,000547
	ttest	0,090869034		0,004971648		0,030143335	

Table 7.3.3: ½ MS heat treatment recovery experiment 1 root lengths measured in mm with 0 h parallel 1 plate 2 excluded.

		WT-0h	*lcmt1*-0h	WT-1h	*lcmt1*-1h	WT-24h	*lcmt1*-24h
Parallel 1	Plate 1	86,45	95,87	83,51	82,21	57,12	38,28
		85,71	98,17	89,16	95,41	48,95	31,19
		99,54	95,37	91,24	90,65	55,1	35,07
		85,77	90,49	82,43	89,5	56,41	33,43
		92,03	79,58	84,62	87,1	54,63	29,84
	Plate 2			85,4	92,77	47,57	45,65
				87,48	100,61	40,82	39,11
				94,41	93,83	49,69	37,98
				90,7	84,56	48,41	31,28
				86,4	90,85	52,52	29,82
	Plate 3	87,5	104,79	87,27	100,24	43,16	28,57
		94,32	104,11	88,69	86,51	50,63	22,59
		88,91	97,79	93,6	91,23	43,89	49,76
		88,57	103,08	87	99,84	45,5	27,05
		92,4	98,74	72,93	90,5	50,87	44,49
Parallel 2	Plate 1	69,37	95,51	91,75	90,92	58,03	43,5
		80,14	89,31	87,4	97	45,6	46,11
		76,24	66,99	90,27	89,53	55,24	13,73

		65,38	76,41	88,25	91,49	56,88	15,03
		78,33	60,52	99,91	61,33	54,93	10,37
	Plate 2	84,69	99,91	45,37	92,79	48,9	47,32
		88,38	89,21	71,84	92,37	51,41	39,41
		79,79	105,52	85,48	97,6	62,66	35,36
		63,7	75,79	77,96	89,48	32,98	38,03
		92,7	97,81	95,22	88,08	52,58	29,11
	Plate 3	78,77	92,34	76,17	95,24	56,48	27,2
		85,96	92,51	85,66	98	59,78	27,71
		94,13	97,44	95,23	99,53	54,21	38,24
		69,95	95,79	88,18	87,06	62,49	28,43
		80,93	93,07	90,64	80,65	61,58	22,81
Parallel 3	Plate 1	85,19	94,42	82,01	85,77	54,91	33,3
		90,4	85,76	87,85	90,52	49,76	45,15
		88,43	87,55	90,69	91,29	46,15	41,24
		90,45	77,49	90,3	102,62	49,14	35,63
		82,79	81,54	95,28	70,79	64,19	36,42
	Plate 2	82,11	85,02	84,33	91,69	49,27	34,73
		85,57	91,5	91,7	87,04	54,49	31,28
		77,79	75,34	81,05	95,21	67,01	31,89
		79,46	73,98	86,61	88,21	59,03	18,91
		88,52	79,28	86,17	89,71	65,52	26,93
	Plate 3	84,58	97,34	91,17	88,1	60,32	36,29
		93,68	84,55	87,09	94,04	54,45	35,77
		92,14	95,25	88,22	87,89	61,25	30,53
		87,2	83,6	89,33	88,52	51,38	31,43
		93,57	80,56	84,5	73,33	49,22	33,89
	mean	84,7885	89,2325	86,45489	90,03578	53,22467	33,108
	SD	8,028975	10,61491	8,388988	7,690284	6,89893	8,733045
	SE	1,269492	1,678365	1,250557	1,1464	1,028432	1,301846
	ttest	0,018954152		0,01881558		9,01023E-21	

Table 7.3.4: ½ MS heat treatment recovery experiment 1 average plant weight measured in gram with parallel 1 plate 2 excluded.

		WT-0h	lcmt1-0h	WT-1h	lcmt1-1h	WT-24h	lcmt1-24h
Parallel 1	Plate 1	0,01802	0,01316	0,0184	0,01148	0,0068	0,00548
	Plate 2			0,01636	0,0139	0,00722	0,00502
	Plate 3	0,013	0,0132	0,01922	0,01256	0,00606	0,00524
Parallel 2	Plate 1	0,00832	0,00996	0,013	0,01286	0,00826	0,00572
	Plate 2	0,01256	0,01846	0,0112	0,01032	0,00772	0,00846
	Plate 3	0,01096	0,0119	0,01192	0,01152	0,00972	0,008
Parallel 3	Plate 1	0,0194	0,0078	0,021	0,014	0,0106	0,0092
	Plate 2	0,0198	0,0136	0,0186	0,0146	0,014	0,0086
	Plate 3	0,025	0,0112	0,0216	0,0136	0,01	0,0068
	Mean	0,0158825	0,01241	0,0168111	0,01276	0,008931	0,006947
	SD	0,0055573	0,003121	0,0039074	0,001419	0,002446	0,00164
	SE	0,0019648	0,0011034	0,0013025	0,000473	0,000815	0,000547
	ttest	0,072809821		0,004971648		0,030143335	

7.4 ½ MS heat treatment experiment 2

Table 7.4.1: ½ MS heat treatment recovery experiment 2 root lengths measured in mm.

		WT-0h	*lcmt1*-0h	WT-1h	*lcmt1*-1h	WT-24h	*lcmt1*-24h
Parallel 1	Plate 1	91,85	94,38	90,43	93,35	47,1	42,98
		93,02	83,73	94,34	94,37	54,76	39,6
		88,48	101,65	91,31	85,83	54,51	39,87
		93,77	93,52	94,56	80,2	49,54	13,24
		86,76	83,75	99,66	72,81	44,87	20,5
	Plate 2	89,4	90,52	90,18	85,32	57,59	43,69
		92,18	89,65	91,8	88,32	52,54	16,71
		88,76	84,96	84,65	72,83	64,77	16,66
		88,28	83,99	94,56	57,78	50,76	37,45
		103	60,57	96,79	82,38	64,51	17,37
	Plate 3	91,21	94,65	83,91	83,04	41,03	30,05
		103,05	87,33	91,39	37,85	45,95	24,82
		98,48	100,62	90,48	89,14	53,36	27
		95,58	62,08	99,35	86,76	52,54	27,52
		95,13	53,44	89,56	60,11	53,2	24,94
Parallel 2	Plate 1	82,59	98,32	80,12	87,86	51,18	31,42
		92,97	96,03	82,5	90,88	61,34	13,25
		92,1	93,1	90,22	62,88	44,65	27,01
		87,61	86,53	89,33	80,87	58,88	22,25
		100	61,52	90,89	56,11	64,27	22,76
	Plate 2	94,21	94,75	91,8	89,55	47,97	32,33
		88,23	87,56	92,98	88,32	63,59	21,11
		86,62	87,38	95,01	50,16	58,48	30,45
		91,9	87,18	68,24	82,07	50,49	38,21

		88,79	89,2	95,35	78,65	35,34	16,63
	Plate 3	93,41	92,3	100,39	77,59	49,82	33,79
		92,67	85,89	93,44	83,68	61,98	21,03
		89,97	85,87	97,19	79,86	52,18	38,57
		89,53	85,24	88,19	73,12	57,16	13,75
		80,24	77,54	96,31	92,88	54,51	16,15
Parallel 3	Plate 1	84,24	85,87	85,46	66,93	57,27	43,72
		90,19	95,01	88,54	82,77	56,02	40,22
		81,53	93,71	79,36	87,24	43,68	39,84
		75,26	85,06	89,27	92,59	52,73	14,07
		89,04	74,96	85,03	73,48	54,46	19,62
	Plate 2	77,11	89,45	90,52	91	51,06	37,13
		81,69	95,15	94,33	91,46	56,54	48,64
		85,61	88,67	88,03	76,2	55,89	22,98
		84,3	81,46	88,78	78,59	58,02	34,06
		90,57	82,02	99,37	92,33	51,52	54,96
	Plate 3	87,34	92,17	88,79	78,3	24,17	15,43
		95,44	94,22	92,56	85,52	39,79	4,28
		89,5	68,67	91,15	95,46	46,83	16,47
		91,05	83,65	88,51	74,92	41,49	6,04
		80,16	77,47	85,96	77,86	46,8	6,84
	mean	89,61822	85,79533	90,45756	79,76044	51,892	26,78689
	SD	5,999002	10,64067	5,957055	12,53783	8,030694	12,11086
	SE	0,894278	1,586217	0,888025	1,869029	1,197145	1,80538
	ttest	0,019322426		7,27107E-07		1,05832E-19	

Table 7.4.2: ½ MS heat treatment recovery experiment 2 average shoot weight measured in gram.

		WT-0h	lcmt1-0h	WT-1h	lcmt1-1h	WT-24h	lcmt1-24h
Parallel 1	Plate 1	0,0108	0,007	0,0138	0,009	0,0062	0,004
	Plate 2	0,0118	0,0084	0,0108	0,0084	0,0066	0,0054
	Plate 3	0,0174	0,0088	0,0126	0,009	0,0056	0,005
Parallel 2	Plate 1	0,0116	0,0096	0,014	0,0086	0,0072	0,004
	Plate 2	0,01	0,0078	0,0172	0,0088	0,0078	0,0048
	Plate 3	0,0108	0,0076	0,0134	0,0078	0,0086	0,0048
Parallel 3	Plate 1	0,0094	0,0064	0,0078	0,007	0,0046	0,005
	Plate 2	0,0098	0,0068	0,011	0,0074	0,0054	0,0052
	Plate 3	0,013	0,009	0,0118	0,0088	0,0044	0,0028
	Mean	0,0116222	0,0079333	0,0124889	0,008311	0,006267	0,004556
	SD	0,0024381	0,0010909	0,0026117	0,000736	0,001425	0,000817
	SE	0,0008127	0,0003636	0,0008706	0,000245	0,000475	0,000272
	ttest	0,000382063		0,000142167		0,003262077	

7.5 Gene expression experiment

Table 7.5.1: C_Q values for the first gene expression run.

		APXS	FSD1	HSP90-1	IRT1	UBC35
	WT-0h	18,63	17,77	25,70	21,38	24,26
	WT-1h	19,25	17,16	16,61	22,94	24,38
	WT-24h	18,86	17,81	19,17	31,07	25,98
	lcmt1-0h	19,29	19,53	26,49	20,26	25,15
	lcmt1-1h	19,61	18,73	16,68	21,97	24,84
	lcmt1-24h	19,68	18,90	20,14	30,31	25,84
WT-0h	SD	0,110604	0,085049	0,169706	0,020817	0,120208
	SE	0,063857	0,049104	0,120003	0,012020	0,085001
WT-1h	SD	0,191572	0,090738	0,155563	0,078102	0,240416
	SE	0,110604	0,052389	0,109998	0,045091	0,170003
WT-24h	SD	0,280535	0,170000	0,193132	0,07000	0,005774
	SE	0,161967	0,09815	0,111504	0,040415	0,003331
lcmt1-0h	SD	0,070946	0,102632	0,190919	0,125033	0,155563
	SE	0,040961	0,059253	0,135001	0,072186	0,109998
lcmt1-1h	SD	0,101489	0,075056	0,298161	0,055076	0,084853
	SE	0,058595	0,043336	0,172143	0,031801	0,059998
lcmt1-24h	SD	0,431335	0,205061	0,305123	0,124231	0,155563
	SE	0,305	0,144999	0,176161	0,071724	0,109998

Table 7.5.2: C_Q values for the second gene expression run.

		APXS	HSP90-1	IRT1	UBC35
	WT-0h	18,53	20,61	20,80	21,56
	WT-1h	19,67	13,36	21,65	21,71
	WT-24h	18,81	15,68	31,87	22,72
	lcmt1-0h	19,54	21,32	19,98	21,69
	lcmt1-1h	20,31	13,70	20,93	21,87
	lcmt1-24h	19,58	16,46	29,19	22,83
WT-0h	SD	0,218251	0,077782	0,100167	0,096437
	SE	0,126007	0,055000	0,057831	0,055678
WT-1h	SD	0,196044	0,260576	0,221886	0,055076
	SE	0,113186	0,150444	0,128106	0,031798
WT-24h	SD	0,130512	0,183576	0,241937	0,073711
	SE	0,075351	0,105988	0,139682	0,042557
lcmt1-0h	SD	0,005774	0,045826	0,030000	0,055076
	SE	0,003334	0,026458	0,017321	0,031798
lcmt1-1h	SD	0,132791	0,111505	0,055076	0,005774
	SE	0,076667	0,064377	0,031798	0,003334
lcmt1-24h	SD	0,388909	0,16563	0,026458	0,238607
	SE	0,275	0,095627	0,015276	0,12776

Table 7.5.3: RQ values relative to WT-0h for the first gene expression run.

	APXS	FSD1	HSP90-1	IRT1
WT-0h	1	1	1	1
WT-1h	7,11E-01	1,66E+00	5,94E+02	3,69E-01
WT-24h	2,82E+01	3,20E+00	3,05E+02	3,99E-03
lcmt1-0h	1,18E+00	5,47E-01	1,08E+00	4,04E+00
lcmt1-1h	7,62E-01	7,71E-01	7,79E+02	9,95E-01
lcmt1-24h	1,46E+00	1,37E+00	1,42E+02	6,15E-03

Table 7.5.4: RQ values relative to WT-0h for the second gene expression run.

	APXS	IRT1	HSP90-1
WT-0h	1	1	1
WT-1h	4,94E-01	6,04E-01	1,65E+02
WT-24h	1,18E+00	6,69E-04	4,39E+01
lcmt1-0h	5,56E-01	1,98E+00	6,85E-01
lcmt1-1h	3,50E-01	1,10E+00	1,45E+02
lcmt1-24h	8,88E-01	5,46E-03	3,24E+01

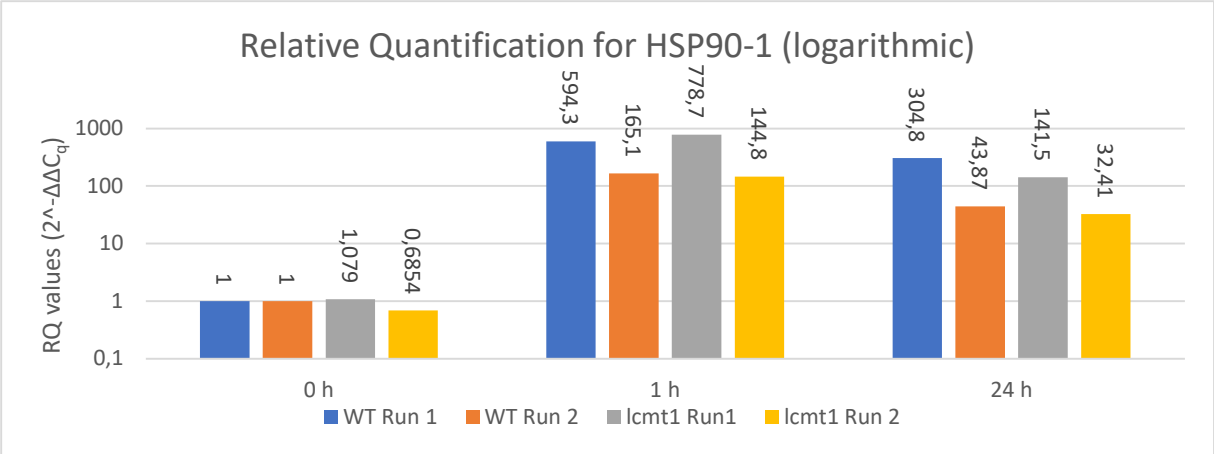

Figure 7.5.1: Comparing the RQ numbers for HSP90-1 between the two runs. Even though the values differed greatly between the two runs, the trend was the same. There was an extremely elevated value for both 1 h and 24 h, but the value was highest in the 1 h group. This was true for both WT and *lcmt1*. A logarithmic scale figure was used here, see figure 3.8.5 for a linear one.

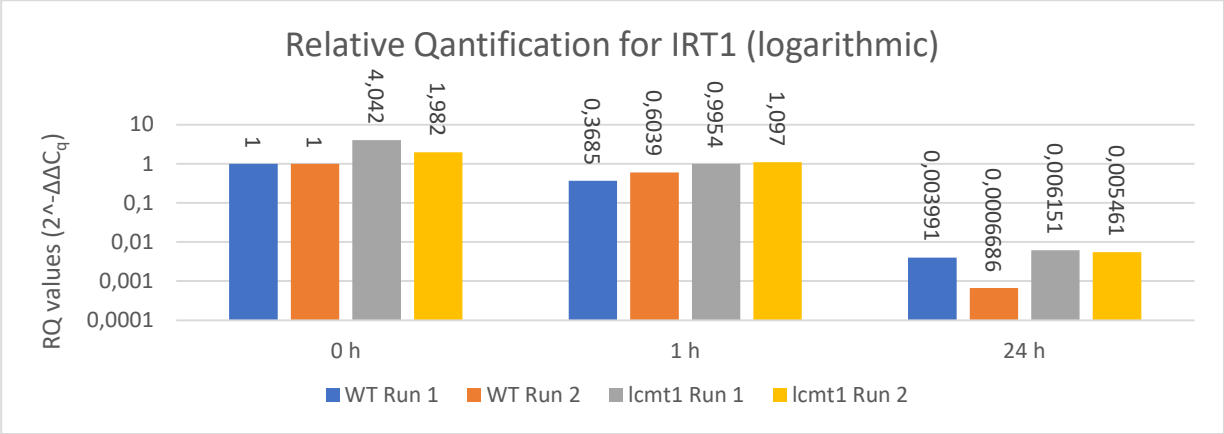

Figure 7.5.2: Comparing the RQ values for IRT1 between the two runs. Even though the values differ greatly between the two runs, the trend was the same. There was an extremely decreased value in the 24 h group. This was true for both WT and *lcmt1*, but even more so for WT. A logarithmic scale figure was used here, see figure 3.8.6 for a linear one.

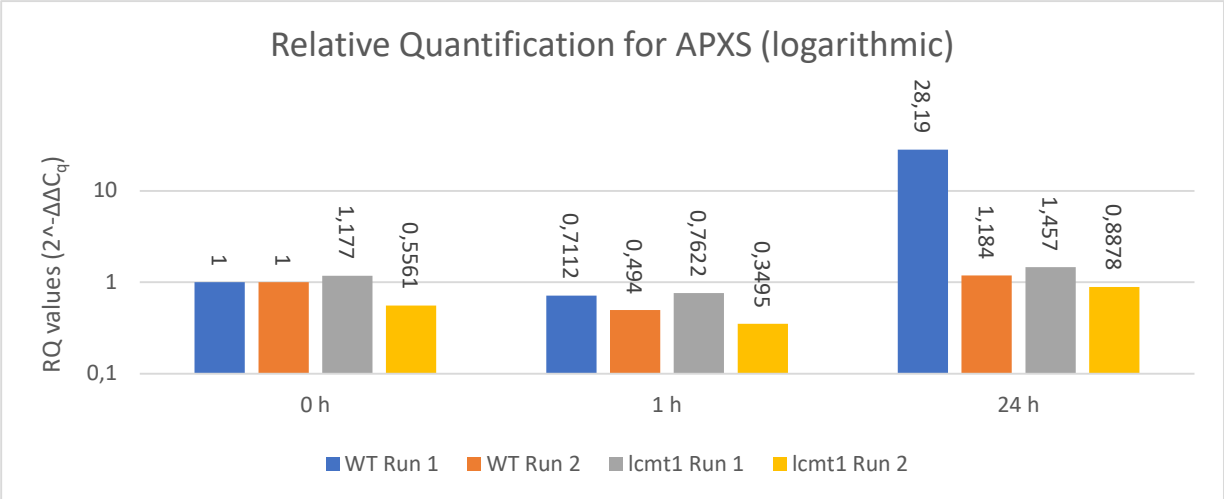

Figure 7.5.3: Comparing the RQ values for APXS between the two runs. They showed the same trend and ignoring the extreme value for WT in the 24 h group, the values were much less extreme than HSP90-1 and IRT1. Both WT and *lcmt1* showed a decreased value in the 1 h group, but an elevated value in the 24 h group compared to the control. A logarithmic scale figure was used here, see figure 3.8.7 for a linear one.