University of Stavanger

Faculty of Science and Technology

MASTER'S THESIS

Study program/ Specialization: Master in Biological Chemistry	Spring semester, 2012..... Open / Restricted access
Writer: Solveig Margret Olafsdottir	..... (Writer's signature)
Faculty supervisor: Professor Cathrine Lillo, UiS External supervisor(s): Dr. Michel Verheul, Bioforsk Vest, Særheim	
Titel of thesis: The influence of nitrogen on compounds and quality of *Arabidopsis thaliana* and tomato	
Credits (ECTS): 60	
Key words: *Arabidopsis thaliana*, MYBL2, CPC, TRY, anthocyanin, nitrogen, tomat, soluble solid content, titratable acidity, yield.	Pages: 110..... Appendix included..... Stavanger, 15.6. 2012 Date/year

Abstract

Part A: The influence of nitrogen on compounds and quality of *Arabidopsis thaliana*

Flavonoids are secondary metabolites in plants that are thought to have beneficial effects on human health, as well as importance for the plants resistance to stress andathogens. MYBL2 and CPC are small MYB proteins that are thought to act as inhibitors in anthocyanin syntesis. TRY is a close homolog of CPC and could also be a candidate in anthocyanin accumulation. The objective of this study was to test of expression of these MYB proteins are influenced by nitrogen depletion and other stress factors, like light intensity. R3 MYB factors TCL, ETC1, ETC2 and ETC3 (CPL3) were also included. The study showed that the MYB proteins MYBL2, CPC and TRY are influenced by nitrogen depletion and light intensity. The results indicate that MYBL2 is an inhibitor but more analysis must be done before making a conclusion. CPC did not follow the same trend as MYBL2 and might therefore not be an inhibitor in anthocyanin synthesis. TRY followed mostly the same pattern as MYBL2 and is therefore more likely to be a negative regulator in anthocyanin accumulation. The expression of the genes *TCL1, ETC1, ETC2* and *ETC3* (*CPL3*) showed interesting trends that could indicate that they have a role in anthocyanin synthesis. Further studies must though be provided before a conclusion can be made.

Part B: The influence of nitrogen on compounds and quality of tomato

The tomato (*Solanum lycopersicum*) is an important source of vitamins, minerals, antioxidants and fibers and is one of the most popular and widely consumed fruit in the world. Taste and appearance are qualities that are important for the consumers and sugars and acids are the main factors contributing to the taste of the tomatoes. It is commonly accepted that the flavor and quality of tomatoes along with other fruits have declined over the last decades. This study is a part of a project where the main goal is to increase the consumer's preference for Norwegian tomatoes. The aim of this part was to see how various nitrogen levels in fertilizers and different light conditions affected the quality and compounds (sugars and acids) in four cherry tomato cultivars. The results showed complex interactions of growth conditions on the fruit quality. Different levels of nitrogen and light did not have much affects on the fruit quality. The main difference was found be between the cultivars.

Abbreviations

ACT8	ACTIN 8
ANS	Anthocyanidin synthase
bHLH	Basic HELIX-LOOP-HELIX protein
cDNA	Complementary deoxyribonucleic acid
CHS	Chalcone synthase
CHI	Chalcone flavonone-isomerase
CO_2	Carbon dioxide
CPC	CAPRICE
CPL3	CAPRICE-like MYB3
Cq	Threshold cycle
DFR	Dihyfroflavonoid 4-reductase
EGL3	Enhancer of GLABRA3
ETC1/2	Enhancer of TRY and CPC 1/2/3
F3'H	Flavonoid 3' hydroxylase
FLS	Flavonol synthase
GL3	GLABRA3
K	Potassium
mRNA	Messenger ribonucleic acid
MS	Murashige and Skoog medium
MYB	MYB family (Myeloblastosis)
MYBL2	MYB-LIKE 2
N	Nitrogen
PAL	Phenylalanine ammonia lyase
PAP1	Production of Anthocyanin Pigment 1
PAP2	Production of Anthocyanin Pigment 2
PCR	Polymerase Chain Reaction
RQ	Relaitve Quantity
SSC	Soluble Solid Content
SSY	Soluble Solids Yield
TAE	Tris-acetate
TCL1	Trichomeless 1
TRY	TRIPTYCHON
TTA	Titratable acidity
TTG1	Transparent Testa Glabra 1
UV	Ultraviolet

WD40	WD40 repeat domain
WT Col	Wild type Columbia
WT Ler	Wild type landsberg erecta
WT WS	Wild type Wasilewskija

List of Contents

Preface and acknowledgement

This master thesis is the final part of my master study at the University of Stavanger where I was a part of the research group of Professor Cathrine Lillo. I also did a part of my work with Dr. Michel Verheul at Bioforsk Vest, Særheim. My report is therefore divided into two parts, the first part presents my work at the University and the second part presents my work at Bioforsk.

I want to thank Professor Cathrine Lillo for her good support and supervision during this master thesis. I am also grateful to Dr. Michel Verheul for his good help and guidance, and giving me the opportunity to work and be a part of his study at Bioforsk Vest.

Special thanks go to Dr. Rune Slimestad for his guidance during the analysis work at Bioforsk. I also want to thank Dugassa Feyissa for his help and patience in the lab at the University. I also want to thank Henk Maessen for his support in the greenhouse at Bioforsk.

Finally, I want to thank my family for their support and encouragement during my master study.

Part A: The influence of nitrogen on compounds and quality of *Arabidopsis thaliana*

1 Introduction

1.1 Background and aim

Flavonoids are secondary metabolites in plants that have received a lot of attention over the last years due to possible beneficial effects on human health, as well as importance for the plants resistance to stress and pathogens. From previous work, it is known that low nitrogen content in the soil/growth medium leads to accumulation of anthocyanins and other flavonoids. Anthocyanin synthesis is regulated by the MYB-bHLH-WDR complex. A specific transcription factor, GL3, seems to be important for the plants response to nitrogen depletion. Other regulatory proteins interact with GL3, like the small MYB proteins MYBLIKE2 (MYBL2) and CPC. MYBL and CPC seem to be involved in the regulation of trichome formation and root hair differentiation, along with TRY which is a close homolog to CPC. It has also been shown that MYBL2 and CPC act as inhibitors in anthocyanin biosynthesis and since TRY is a close homolog of CPC, it could also be a good candidate in anthocyanin accumulation. To our knowledge TRY has not been tested previously in relation to anthocyanin synthesis.

In the present work we will test if expressions of these inhibitory MYB proteins are influenced by nitrogen depletion and other stress factors, like light intensity. Anthocyanin concentration and the transcript level (mRNA) of these genes will be measured in leaves of plants growing on complete nutrient solution, as well as in plants deprived of nitrogen. Various knockout mutants will be included in the experiment to achieve a better understanding of interaction and involvement of different regulators.

1.2 *Arabidopsis thaliana*

Arabidopsis thaliana is a small dicotyledonous species that is widely used as a model organism in plant biology. It is a member of the Brassicaceae or the mustard family. *A. thailana* is related to important crop plants such as cabbage and radish, but is itself of no major agronomic significance. It does, however, offer important advantages for basic research in genetics and molecular biology. The plant has a fast lifecycle of approximately 48 days and is easily grown in indoor growth chamber or

greenhouses. It has a relatively small, genetically tractable genome that can be manipulated easily through genetic engineering. Crop plants, like cabbage or radish, have larger genomes which often poses challenges to the researcher. Arabidopsis makes it easier to test hypothesis quickly and efficiently, and the knowledge we gain from this model plant can be used to improve plants of economic and cultural importance (Tair database; nsf).

1.3 Flavonoids

Flavonoids constitute a sub-group of phenylpropanoids and consist of about 10.000 members. They include two main groups, the 2-phenylchromans (the flavonoids including flavanones, flavones, flavonols, flavan3-ols and anthocyanins) and the 3-phenylchromans (the isoflavonoids which comprise the isoflavones, isoflavans and pterocarpans) (Dixon et al. 2010; Olsen et al. 2008).

Flavonoids are secondary compounds which contribute to the color and taste of fruits and vegetables. They can act as attractants for pollinators, protect the plant against UV irradiation and pathogens, and are believed to have health-beneficial effects (Lillo et al. 2008). The concentration of flavonoids generally increases when plants are exposed to stress factors like pathogens or UV irradiation. Although not well understood, it has been documented that plants show the same response when given limited nutrition, especially when deprived of nitrogen or phosphorous. Manipulation of these macronutrients might therefore be used to control the accumulation of wanted compounds in plants (Lillo et al. 2008; Olsen et al. 2009; Misson et al. 2005; Lea et al. 2007).

Because of its potential health-beneficial effects on humans, flavonoids, including anthocyanins, have been in focus in medical and agricultural studies. Anthocyanins have been linked to potential health effects against diseases like cancer. Several studies have been done on crop fruits, including tomato. It has for example been shown that the concentration of anthocyanins in tomato plants increase when the plants are deprived of nitrogen (Løvdal et al. 2010). Although anthocyanin concentration was not as high in the eatable part of the plant, the fruit, nutrient stress can be a way of directing plants to accumulate this compound in vegetative crops. A combination of genetic engineering and environmental factors can therefore be used as tools to change the content of desirable compounds in leafs or fruits to improve the quality of fruits and vegetables. A high level of anthocyanins might also lower the need for pesticide treatments (Løvdal et al 2010; Lea et al. 2007).

1.4 The flavonoid pathway

Flavonoids are synthesized via the phenylpropanoid pathway (Fig. 1). PAL genes (Phenylalanine ammonia lyase) control the flux of primary metabolism into the flavonoid pathway from the shikimate pathway by catalyzing the conversion of L-phenylalanine to cinnamate. These PAL genes may give rise to different metabolic pools that are channeled into different pathways (Lillo et al., 2008). Several enzymes catalyze different metabolism in the flavonoid pathway, *CHS*, *CHI*, *F3H*, *F3'H*, and *FLS* (early genes) being the first genes to be activated. Later, *DFR* and *ANS* are avtivated (late genes). They catalyse the formation of leucocyanidin and leucoanthocyanidin in anthocyanin biosynthesis (Lillo et al. 2008;). The early genes and especially the late genes are strongly induced by nutrient deficiency (Scheible et al. 2004; Misson et al. 2005; Morcuende et al, 2007).

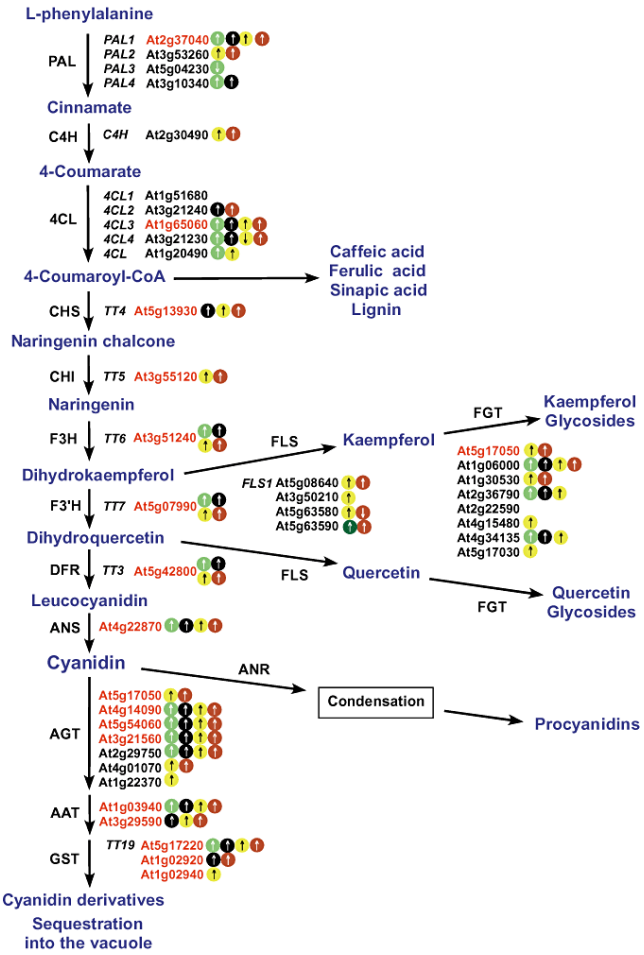


Figure 1. Simplified scheme of the flavonoid and phenylpropanoid pathway in *Arabidopsis thaliana*. Key enzymes marked are PAL (phenylalanine ammonia.lyase), CHS (chalcone synthase), FLS (flavonol synthase) and ANS (anthocyanidin synthase). This scheme is taken from Olsen et al. (2007).

1.5 Regulation of anthocyanins

Synthesis of anthocyanins seems to be the same throughout the plant kingdom. It has been found to be regulated in all higher plants by MYB and bHLH transcription factors (Ramsay et al. 2005). R2R3 MYB transcription factors (PAP1, PAP2) are known to form complexes with bHLH factors (EGL3, GL3, TT8) and interact with a WD40 protein (TTG1) (Lepiniec et al. 2006). Yeast two-hybrid studies have also shown that EGL3/GL3 interact with both TTG1 and PAP1/2, forming a ternary complex (Zhang et al. 2003). This complex appears to be important for activating certain steps in the flavonoid pathway. It is known to activate both DFR and ANS promoters (Pelletier et al. 1997; Zhang et al. 2003; Gonzalez et al. 2008).

It has been shown that *PAP1/2*, *EGL3* and *GL3* expression is strongly induced by nitrogen depletion (Feyissa et al. 2009). Zhang et al. (2003) and Gonzalez et al. (2008) showed that EGL3 has a greater role than GL3 in anthocyanin synthesis. They meant that inactivation of GL3 had no effect as long as EGL3 was present. The contrast has also been shown where *GL3* transcript levels were strongly enhanced in response to nitrogen depletion, indicating that GL3 is essential in accumulation of anthocyanins (Lea et al. 2007). These results were supported by a study made on *egl3* and *gl3* loss-off-function mutants, where it was observed that anthocyanin accumulation was very low in the *gl3* mutant indicating that GL3 has a an important physiological function in the synthesis of anthocyanins and cannot be replaced by EGL3 (Feyissa et al. 2009).

Arabidopsis MYB-LIKE2 (MYBL2) is a R3 MYB protein that has been shown to be a negative regulator of anthocyanin synthesis (Dubos et al. 2008; Matsui et al. 2008). Dubos et al. (2008) showed that overexpression of *MYBl2* inhibits anthocyanin accumulation, whereas knockout of *MYBL2* enhances anthocyanin synthesis. Dubos showed that expression of *MYBL2* was reduced in rosette stage after being exposed to highlight intensity for 51 h. Other stress effectors, like nitrogen depletion, were not included in their work.

According to Wang et al. (2008), there are six single-repeat R3 MYB factors in the *Arabidopsis* genome. They are: CPC, TRY, TCL1, ETC1, ETC2 and ETC3 (CPL3). These factors have been shown to be involved in regulation of trichome formation and root hair differentiation, and are known to interact with GL3 (Wada et al. 1997; Wang et al. 2008).

CPC is a small MYBL protein known to be expressed at a very low level in leaves. Zhu and coworkers (2009) found that CPC is a negative regulator of anthocyanin biosynthesis. They used WT WS, *35S cpc* overexpression plants and *cpc*-1 knockout plants in their experiment. They showed that *35S cpc* plants accumulated less anthocyanins than WT when exposed to nitrogen depletion stress, and that

this was not due to an increase in nutrient uptake because of more root hair production. Anthocyanin transcription genes were shown to be down-regulated in the *35S cpc* plants. *cpc-1* knockout plants accumulated more anthocyanins than WT, even though they were not as affected as *35S cpc*. They proposed that CPC competes with the R2R3 MYB-transcription factor PAP1/2 to GL3/EGL3.

A close homolog of CPC is TRY, which is known to be a negative regulator of trichome formation (Wang et al. 2008). According to TAIR database T*RY* is expressed in leaves at a higher level than *CPC*. This could indicate that TRY, as CPC, may be an inhibitor in anthocyanin accumulation and might even be a better candidate for being of physiological importance in that process.

2. Materials and methods

2.1 Plant Material

The following lines were used in this study:

WT Ler

egl3 – a deletion identified.

gl3 – codon 378 mutated to a stop codon.

Seeds were kindly provided by John Schiefelbein (University of Michigan, Ann Arbor, MI, USA).

WT WS

35S cpc – over- expression line. CPC cDNA clone subcloned into the vector pMAT 137 – Hm between Xba I and Kpn I sites under control of the 35S promoter of the cauliflower mosaic virus and introduced into *A. thaliana.*

cpc-1 – T-DNA insertion mutant.

Seeds were kindly provided by NASC and donated by Takuji Wada at Riken.

WT Col B

try – Vacuum infiltration with *Agrobacterium turnefaciens* vector pROK2.

mybl2 – T-DNA insertion mutant.

try was kindly provided by NASC and donated by David Marks, University of Minnesota. *mybl2* was provided by Masaru Takagi, National Institute of Advanced Industrial Science and Technology, Tokyo.

2.2 Seeds sown on rock wool

Plants sown on rock wool were grown with full Hoagland solution containing 15 mM KNO_3 until they had reached rosette stage in their lifecycle (see Tair database). This takes usually 3 - 4 weeks. After that they were treated with and without nitrogen until anthocyanin accumulation could be seen.

The mutants *35S cpc* and *cpc-1* are in *A. thaliana* WS background, and were therefore sown with WT WS.

The mutants *try* and *mybl2* are based on *A. thaliana* Columbia (WT Col), and these ecotypes were therefore sown together.

2.2.1 WT WS, *35S cpc*, *cpc-1*, WT Col, *try* and *mybl2* - Experiment 1

Seed of *Arabidopsis thaliana* ecotype WS, Col, *35S cpc*, *cpc-1*, *try* and *mybl2* were sown on rock wool. WT WS, *35S cpc* and *cpc-1* were sown together in a plastic container, and WT Col, *try and mybl2* were sown together in a different plastic container. Each line was sown on 14 rock wool cubes with full Hoagland solution containing 15 mM KNO_3 (Table A24 in Appendix A). The plastic containers were covered with aluminum folium and placed in a dark room at temperature 4°C for 3 days. The plants were transferred to a growth chamber at temperature 22°C. Each line was grown under continuous light conditions. Light was provided by fluorescent lamps (Osram L58W/77). The flux density (PPFD) was 100 µm $m^{-2}s^{-1}$. Half of the plants were grown in continuous light - full strength (six lamps turned on) and the other half in continuous light-half strength (three lamps turned on).

Continuous light-full strength: The plants were watered with a complete Hoagland solution when needed. When the plants had reached rosette stage (after 24 days) they were ready to be treated with and without nitrogen. The rock wool cubes were washed with tap water to make sure that no nitrogen was available for the plants that would be deprived of that compound. The seedlings were divided into two groups. Half of the seedlings were watered with Hoagland solution containing nitrogen while the other half was treated with Hoagland solution without nitrogen (Table A25 in Appendix A). The plants were treated for 5 days. The plants were then harvested and the leaves placed in liquid nitrogen (-200°C). The samples were then kept frozen at -80°C before crushed and measured for anthocyanins. Three parallels were measured for each ecotype treated with and without nitrogen.

Continuous light-half strength: These plants had to be moved to a growing chamber with photoperiod 16 h light/8 h dark because they did not accumulate anthocyanins after being exposed to continuous light – half strength for 10 days. The reason could be that the temperature in the room was too high leading to less effect in the plants. The plants were treated without nitrogen for 5 days before harvested and kept frozen at -80°C until the amount of anthocyanins was measured. Three parallels were measured for each ecotype.

It was decided not to grow plants in continuous light – half strength again. In the rest of this thesis, plants that were grown in continuous light were exposed to full light strength.

2.2.2 WT WS, *35S cpc*, *cpc-1*, WT Col, *try* and *mybl2* - Experiment 2

Experiment 1 was repeated and the plants were sown on rock wool like before and placed in a growth chamber at 22°C, half of them growing in continuous light while the other half was placed in a 16 h light/8 h dark regimen. The plants growing in continuous light were treated for 5 days, while the other plants were treated for 7 days.

2.2.3 WT WS, *35S cpc*, *cpc-1*, WT Col, *try* and *mybl2* - Experiment 3

The experiment with the plants growing in continuous light was repeated to see if similar results were gained. The procedure was the same as before. The plants were treated with and without nitrogen for 5 days.

2.2.4 WT Ler, egl_3 and gl_3

Seeds of *Arabidopsis thaliana* ecotype Ler, egl_3 and gl_3 were sown on rock wool. Each line was sown on 14 rock wool cubes with full Hoagland solution containing 15 mM KNO_3. The plastic containers were covered with aluminum folium and placed in a dark and cold (4°C) room for 2 days. The plants were then transferred to a growth chamber at temperature 22°C in a 16-h light/8-h dark regimen. The plants were watered with a complete Hoagland solution as a nitrogen source when needed. After 4 weeks, the rock wool cubes were washed with tap water and seedlings of each line were divided into two groups for further treatment. Half of the seedlings were watered with complete Hoagland solution while the other half was treated with Hoagland solution without nitrogen. Half of the plants received the treatment for three days while half of them were treated for 5 days before harvested and analyzed by real-time PCR.

2.2.5 WT WS and WT Col

WT WS and WT Col were sown together on a rock wool to be analyzed by real-time PCR. Each line was sown on 14 rock wool cubes with full Hoagland solution containing 15 mM KNO_3. The plastic containers were covered with aluminum folium and placed in a dark and cold (4°C) room for 3 days. The plants were placed in 16 h day/8 h dark rhythm and in continuous light. They were treated with

and without nitrogen for 7 days (plants in 16 h day/8 h dark rhythm) and 5 days (plants in continuous light).

2.3 Seeds sown on agar

Plants sown on agar were harvested at seedling stage, when anthocyanin accumulation could be detected (see Tair database).

2.3.1 WT Ler, *egl$_3$* and *gl$_3$*

Sterilized seeds of *Arabidopsis thaliana* ecotype Ler, *egl$_3$* and *gl$_3$* were sown on ½ MS + N 3% sucrose, ½ MS - N 3% sucrose, ½ MS + N 1% sucrose and ½ MS - N 1% sucrose media (see Appendix A). The dishes were placed at 4°C for 2 days. The plants were transferred to a growth chamber at 22°C under continuous light conditions and treated with and without nitrogen for 7 days.

The seeds were sterilized with 1 ml 1% (w/v) Ca – hypochlorite + 1 drop of Tween in 9 ml 96% ethanol. After 5 minutes in the solution, the supernatant was pipette off and the seeds were washed twice with 1 ml of 96% ethanol.

The plants were harvested and further analyzed by real-time PCR. The plants were picked from the agar and placed on a Petri dish containing filter soaked with sterile water. The water was used to prevent the plants from drying during analysis. The roots were cut from the plants and all plant material was put in liquid nitrogen to keep it frozen.

2.3.2 WT WS, *35S cpc*, *cpc-1*, WT Col, *try* and *mybl2*

Sterilized seeds of *Arabidopsis thaliana* ecotype WS, Col, *try*, *35S cpc* and *cpc – 1* were sown on ½ MS + N 1% sucrose and ½ MS - N 1% media. Wt Col and mybl2 were also sown on ½ MS + N 1% sucrose and ½ MS - N 1% media containing 0.5 mM glycine. The dishes were placed at 4°C for 3 days. The Petri dishes were transferred to a growth chamber at 22°C in a 16 h light/8 h dark regimen. The plants were very small and there were not much visual signs of anthocyanin accumulation. For that reason, the plants were moved into a growth chamber with continuous light. After 4 days, the plants were examined with microscope and anthocyanin accumulation compared between ecotypes and treatments.

The plants were sterilized the same way as WT Ler, *egl$_3$* and *gl$_3$* (see 2.3.1 above).

2.4 Anthocyanin measurement

Anthocyanin measurement was performed on six lines of *Arabidopsis thaliana*, WT WS, *35 cpc, cpc-1*, WT Col, *try* and *mybl2*. Plant tissue (50 mg) was harvested (without roots) and kept frozen in liquid nitrogen. The samples were then stored at -80°C until measured for anthocyanin concentration.

To prepare the samples for anthocyanin measuring, frozen plant tissue was put in a mortar and crushed into a fine powder while kept frozen. The powder was put in an Eppendorf tube and 300 µl of 1 % (v/v) HCl in methanol was added. The samples were placed at 4°C for over-night shaking. The next day, 200 µl of distilled water was added and the tubes shaken. Then, 500 µl of chloroform was added and the tubes were shaken again. The tubes were then spun at 13 000 rpm for 2 minutes. The upper layer (400 µl) of each sample was put in a clean Eppendorf tube and 600 µl of 1 % (v/v) HCl in methanol added. The tubes were again spun at 13 000 rpm for 2 minutes to settle particles.

Spectrophotometer (AnalytikJena SPECORD 200) was used to measure anthocyanin. The absorbance was measured at 530 and 657 nm. Relative concentration of anthocyanins were calculated as $absorbance_{530}$ - $absorbance_{657}$ and concentration per gram was calculated as $absorbance_{530}$ - $absorbance_{657}$/gram plant tissue.

2.5 Real-time PCR

Before gene expression could be analyzed in real-time PCR, samples had to be prepared. The ribonucleic acid (RNA) was first isolated and the quality measured. Finally, complimentary DNA (cDNA) was made and used in the real-time PCR running.

2.5.1 RNA isolation and quality check

To prepare samples for RNA isolation, plant material was put in a mortar filled with liquid nitrogen. The plants were crushed into a powder while kept frozen. For RNA isolation it is desirable to get 100 mg plant material. The plant material was kept frozen in -80°C until RNA isolation.

The RNA isolation was carried out by using Quiagen RNeasy plant mini kit. The manufacturer's instructions were used. When the isolation was completed all samples were kept frozen at 80°C to prevent them from degrading.

The quality of RNA was tested by Nanodrop (Thermo Scientific Nanodrop 2000) and gel electrophoresis.

Nanodrop measures RNA (A260) concentration and the purity of the sample (260nm/280nm ratio). The ratio of absorbance at 260 and 280 nm should be around 2.0 to be accepted as pure RNA. 1 µl of sample was used to measure these factors. Nuclease free water was used as a reference.

The samples were also run on a 1% TAE agarose gel to check the quality of the RNA. Half a gram of agarose was added to 50 ml of TAE buffer, dissolved while heated and poured into a mould containing 15 wells. 3 µl of Bioline Hyperladder I was used as a reference mixed with 1.5 µl Biotium red and 1.5 µl of loading buffer. Each sample was mixed with the same amount of Biotium red and the loading buffer.

2.5.2 Making cDNA

cDNA was synthesized using High Capacity cDNA Archive kit (Applied Biosystems). Each sample (200 µl) was made from 100 µl 9.2 ng/µl RNA and 100 µl 2x RT master mix (table 1). The protocol following the kit was used in this process. The samples were kept frozen during the procedure.

The samples were loaded to a PCR (Labnet multigene II) and a cDNA synthesizing program was used (Table 2).

Table 1. 2x RT master mix for cDNA synthesis.

	Pr. Reaction (µl)
10x RT buffer	2,0
25x dNTP mix (100 nM)	0,8
10x RT random primers	2,0
Reverse Transcriptase	1,0
Nuclease free water	4,2
Total	10,0

Table 2. Temperature profile for cDNA synthesis

Temperature (°C)	**Time (minutes)**
25	10
37	120
85	5
4	∞

2.5.3 Real-time PCR analysis

Real-time PCR was performed by using Applied biosystems 7300 Fast Real-Time PCR System. The manufacturer's instructions were used. Primers were predesigned TaqMan gene expression assays obtained for the following genes (TaqMan identification number is given in brackets):

EGL3 A1g63650 (At02217883_1g)

GL3 At5g41315 (At02327731_g1)

MYBL2 At1g71030 (At02227306_g1)

CPC At2g46410 (At02263730_g1)

TRY At5g53200 (At02321066_g1)

DFR At5g42800 (At02314550_g1)

CPL3 At2g33540 (At02207399_g1)

ETC1 (At02258450_g1)

ETC2 At2g30420 (At02252050_g1)

TCL1 At2g30432 (At02610228_m1)

ACT8 At1g49240 (At02270958_gH)

Ubq At3g02540 (At02163241_g1)

ACT8 and *Ubq* were used as endogenous controls.

The reaction volume was 20 µl, containing 2 µl of cDNA, 11 µl of master mix and 7 µl of nuclease free water. Standard cycling conditions (2 min at 50 °C, 10 min at 95°C and 40 cycles altering between 15 s at 95°C an 1 min at 60°C) were used for product formation.

Threshold cycles (Cq values) and relative quantity (RQ values) were used to determine the expression of the genes. Cq levels indicate the number of cycles required for the fluorescent signal to exceed background level. The lower the Cq level the greater the amount of target nucleic acid is in the sample. Gene expression for each sample was calculated on three analytical replicates. Relative quantity of any gene is given as a fold change relative to WT grown on complete medium. Gene expression is also given relative to endogenous controls. Cq values are usually complementary to RQ values, that is, the lower the Cq values are the higher RQ values become.

2.6 Statistical analysis

Pearson correlation (Pearson's r) was calculated to find the relationship between RQ values.

Statistical significance for the difference between anthocyanin concentrations was tested by Mann-Whitney test. The confidence level was set to 95%.

3 Results

3.1 Anthocyanin measurement – seedling stage

3.1.1 WT WS, *35 cpc*, *cpc-1*, WT Col, *try* and *mybl2*

The seeds were sown on Petri dishes with and without nitrogen in continuous light. Low anthocyanin accumulation could be seen in the plants deprived of nitrogen. WT Col and *mybl2* that received glycine were bigger than the plants grown without it but their roots were very small (Fig. 2).

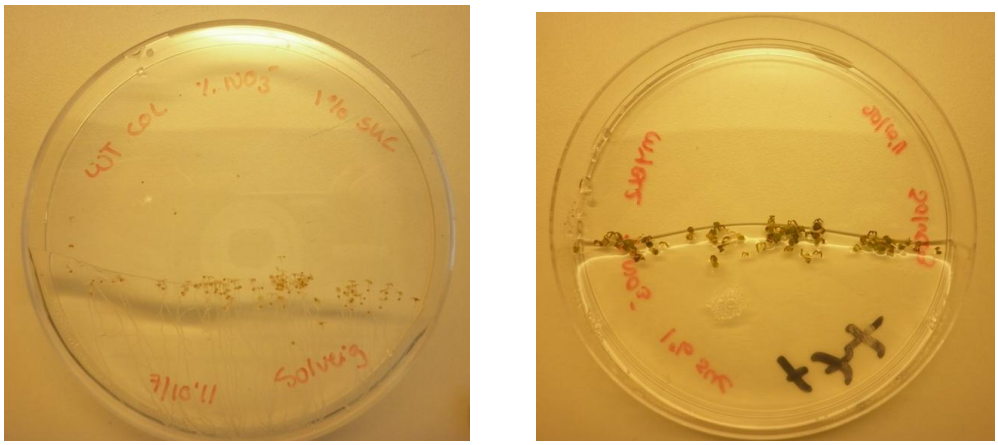


Figure 2. WT Col and *mybl2* deprived of nitrogen. The mutant *mybl2* (to the right) was grown with glycine and developed much smaller roots than WT Col (to the left) which did not receive glycine.

Because of the low anthocyanin accumulation, it was decided to evaluate the amount of anthocyanin production by a microscope (Table 3).

Table 3. Comparison of anthocyanin accumulation in plants (ecotypes WT WS, *35 cpc*, *cpc-1,* WT Col, *try* and *mybl2)* sown on Petri dishes. The signs (- and +) indicate how much anthocyanin accumulation was analysed visually in the plants. The numbers specify how many Petri dishes were evaluated in each category.

Ecotype	**Anthocyanin accumulation**				
	-	(+)	+	++	+++
WT WS -N	2	6	1		
***35S cpc* -N**	6	3			
***cpc-1* -N**	2	7			
WT COL -N		5	4		
***try* -N**			4	1	4
WT COL -N		5	3	2	
***mybl2* -N**		8	1		1

3.2 Anthocyanin measurement - Rosette stage

Seeds of WT WS*, 35S cpc, cpc-1*, WT col, *try* and *mybl2* were sown on rock wool and treated with and without nitrogen in continuous light and 16 h day/8 h night rhythm. The plants were then measured for anthocyanin concentration. The experiment was performed three times in continuous light and twice in day/night rhythm.

It was observed in all experiments that the plants treated with nitrogen became green and looked healthy. Little or no anthocyanin accumulation could be detected visually. On the other hand, plants treated without nitrogen had darker leaves indicating anthocyanin production. This is illustrated more closely in the pictures below (Fig. 3 and 6). It was also noticed that leaves that accumulated anthocyanins showed more color change abaxial than adaxial. Plants, grown in continuous light grew faster than plants located in the 16 h light/8 h dark rhythm growing chamber. They also accumulated anthocyanins earlier.

3.2.1 Continuous light:

WT WS, *35 cpc, cpc-1,* WT Col, *try* and *mybl2* were grown in continuous light and treated with and without nitrogen for 5 days.

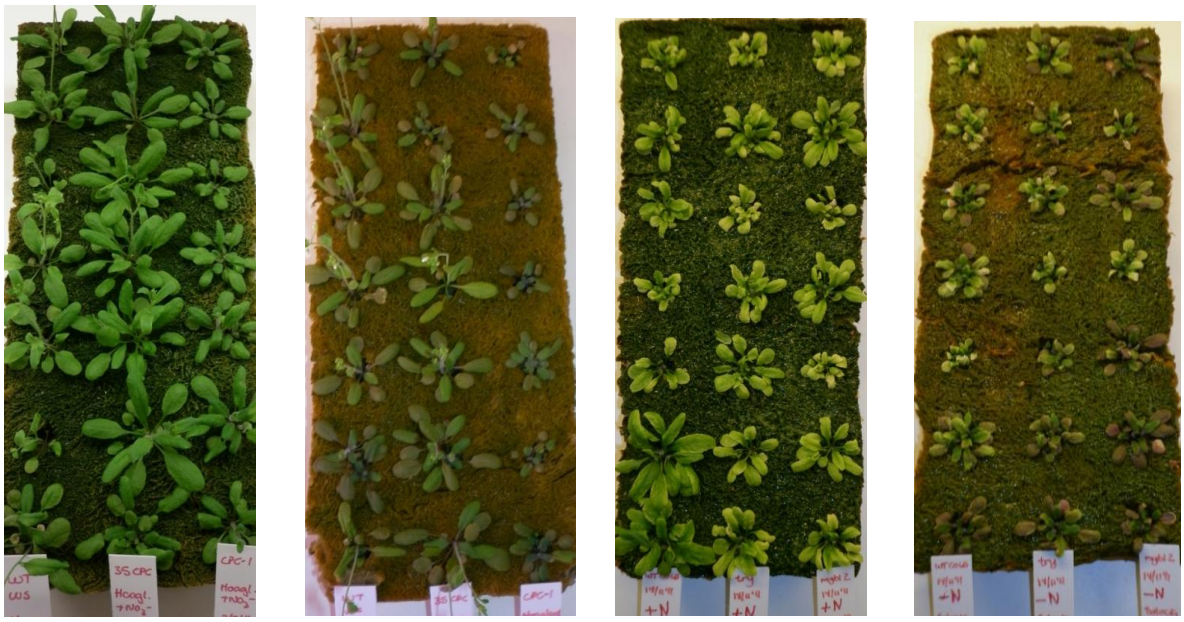


Figure 3. *Arabidopsis thaliana* ecotype WT WS, *35S cpc*, *cpc-1* WT Col, *try* and *mybl*2 were sown on rockwool and grown in continuous light. The plants in photo 1 and 3 were treated with nitrogen while the plants in photo 2 and 4 were deprived of nitrogen.

The plants were measured for anthocyanin concentration. All three experiments gave similar results and were therefore combined (Fig. 4 and 5 and Table A6 in Appendix A). Data for the three experiments is found in tables A1, A3 and A5 in Appendix A. Relative anthocyanin concentration in the mutants to the concentration in WT –N was also calculated and can be seen in Figure 4 and 5. Data for the relative concentration is in Table A8 in Appendix A.

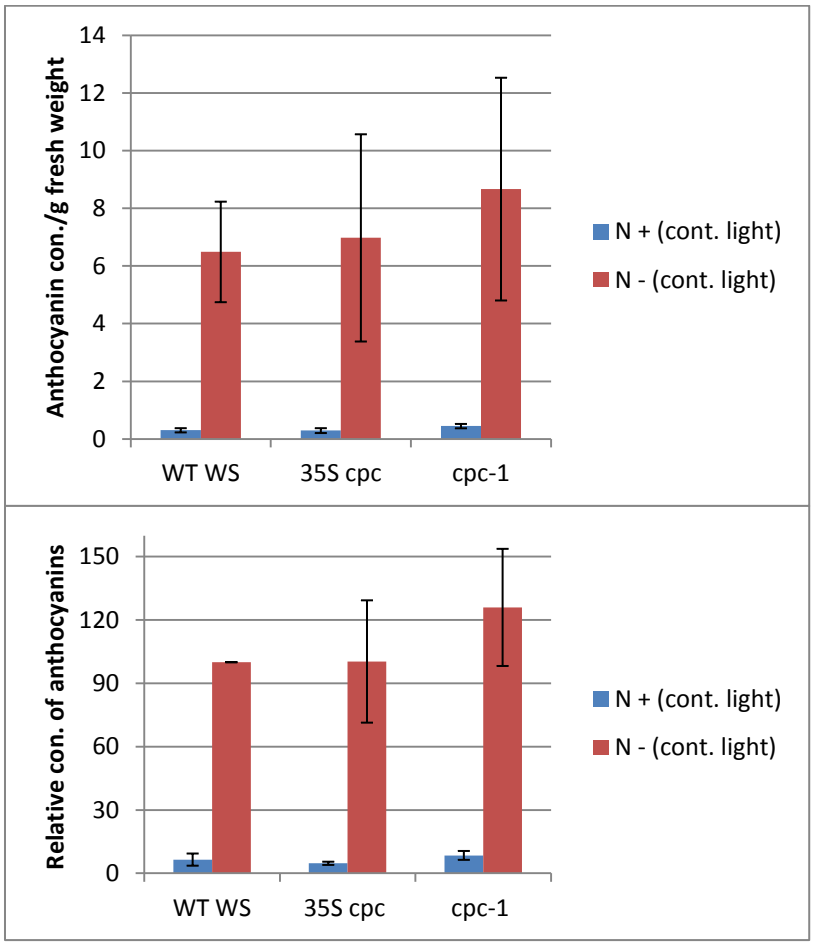


Figure 4. Anthocyanin con./g fresh weight and relative con. of anthocyanins in WT WS, *35 cpc* and *cpc-1* at rosette stage. The plants were grown in continuous light and treated with and without nitrogen for 5 days. Data presented are means of three experiments, with standard error.

Plants deprived of nitrogen accumulated more anthocyanins than plants watered with full Hoagland solution (Fig. 4). The mutants produced more anthocyanins than wild type *cpc -1* having the highest concentration. The difference between mutants and wild type was not significant (Table 4).

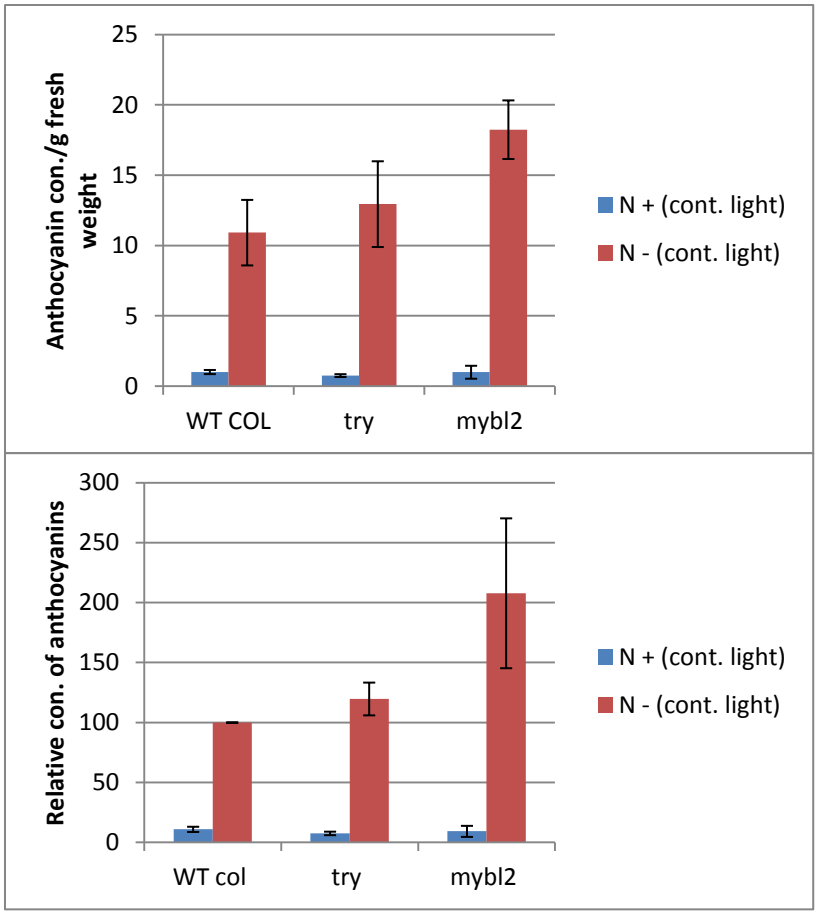


Figure 5. . Anthocyanin con./g fresh weight and relative con. of anthocyanins in WT COL, *try* and *mybl2* at rosette stage. The plants were grown in continuous light and treated with and without nitrogen for 5 days. Data presented are means of three experiments, with standard error**.**

As can be seen in Figure 5, there was a difference in anthocyanin concentration between WT Col and the mutants. The concentration in *try* was not significant different from wild type, when anthocyanins were measured per gram. Significant difference was seen when relative concentration was measured, $p<0.05$ (Table 4). The difference between wild type and *mybl2* was higher and was found to be significant, $p<0.05$ (Table 4).

Table 4. Mann-Whitney was used to test the difference in measured anthocyanin concentration. The confidence level was set to 95%. Data is for plants grown in continuous light and treated for 5 days.

Ecotype	**Significant difference *; p<0,05, **; p<0,01, ***; p<0,001**	**Significant difference in relative anthocyanin con. *; p<0,05, **; p<0,01, ***; p<0,001**
WT WS*35S cpc	n.s.	n.s.
WT WS*cpc-1	n.s.	n.s.
WT COL*try	n.s.	*
WT COL*mybl2	**	*

3.2.2 16 h light/8 h dark rhythm

The same pattern was found for plants grown in 16 h day/8 h night rhythm as in plants grown in continuous light when analyzed visually. Plants treated with nitrogen were green while plants deprived of nitrogen became darker because of anthocyanin accumulation (Fig. 6).

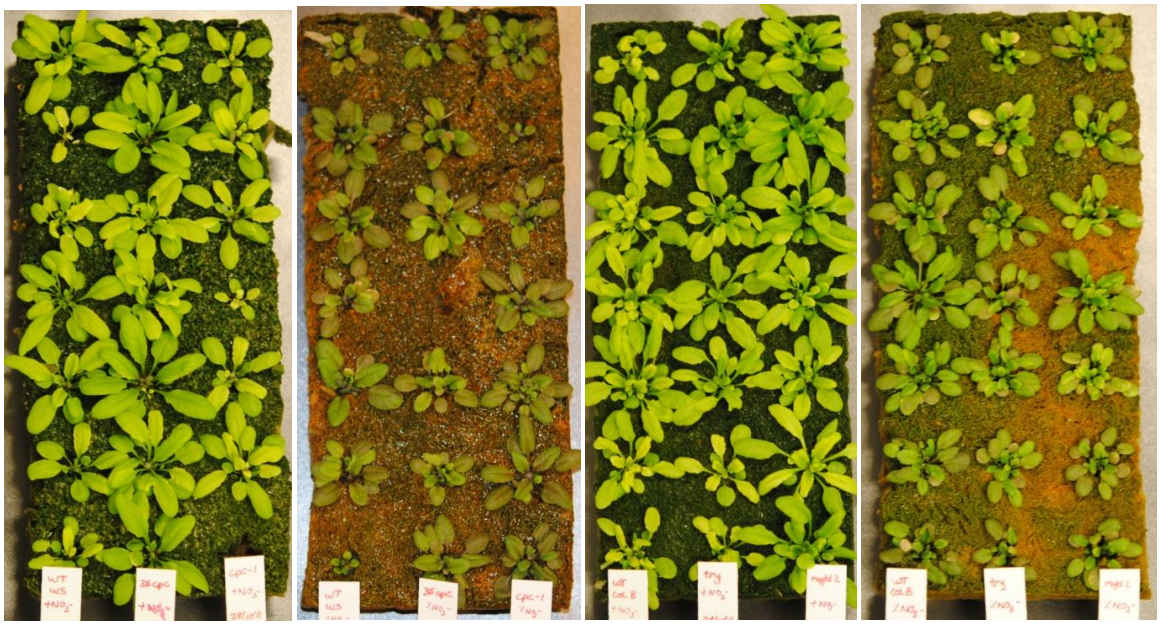


Figure 6. *Arabidopsis thaliana* ecotype WT Col B and *try* sown on rock wool and grown in 16 h day/8 h light rhythm. The plants were treated with and without nitrogen for 5 and 7 days. Plants in photos 1 and 3 were treated with nitrogen while plants in photos 2 and 4 were deprived of nitrogen.

The plants were measured for anthocyanin concentration. Both experiments gave similar results and were therefore combined (Table A7 in Appendix A). Data for the two experiments can be seen in Tables A2 and A4 in Appendix A. Anthocyanin concentration/g and relative anthocyanin concentration of the mutants to the concentration in WT was also calculated and can be seen in Figure 7 and 8. Data for the relative concentration is in Table A9 in Appendix A.

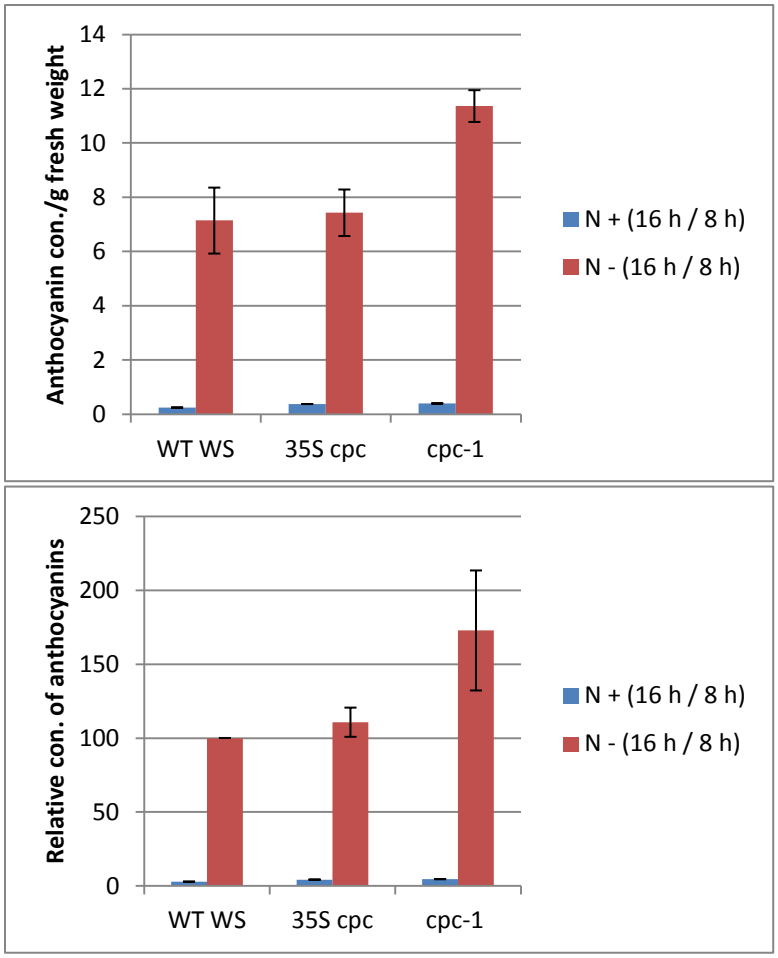


Figure 7. Anthocyanin con./g fresh weight and relative con. of anthocyanins in WT WS, *35S cpc* and *cpc-1* at rosette stage. Plants were grown in 16 h/8 h day rhythm and treated for 5 and 7 days. Data presented are means of two experiments for minus nitrogen and one experiment in plus nitrogen, with standard error.

The anthocyanin concentration was higher in plants that were deprived of nitrogen than in plants that received nitrogen. The concentration was similar between WT WS and *35S cpc* but the difference was much clearer between wild type and *cpc-1* (Fig. 7). The difference in concentration between wild type and *cpc-1* was significant, $p<0,05$ (Table 5).

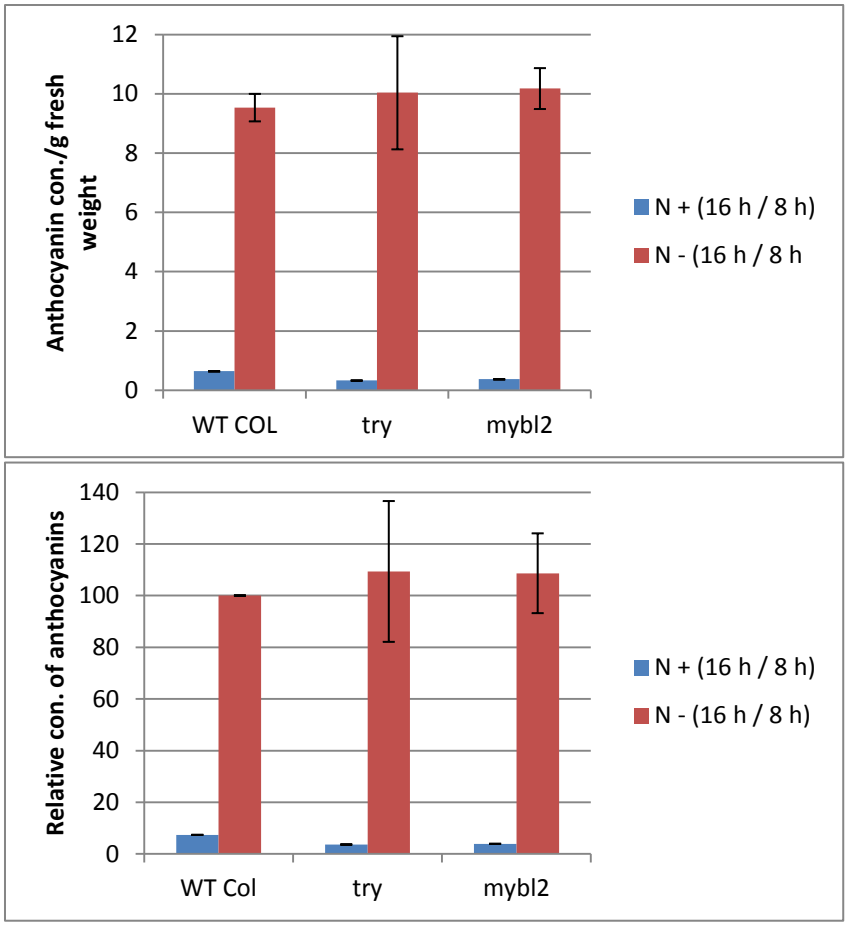


Figure 8. Anthocyanin con./g fresh weight and relative con. of anthocyanins in WT COL, *try* and *mybl2* at rosette stage.Plants were grown in 16 h/8 h day rhythm and treated for 5 and 7 days. Data presented are means of two experiments for – N but only one experiment was experiment for + N, with standard error.

As is illustrated in Figure 8, plants starved with nitrogen produced more anthocyanins than plants that received nitrogen. The difference between wild type and the mutants was not severe and was not found to be significant (Table 5).

Table 5. Mann-Whitney used to test the difference in measured anthocyanin concentration. The confidence level was set to 95%.. Plants were grown in 16 h light/8 h dark rhythm.

Ecotype	**Significant difference in measured anthocyanin con. *; p<0,05, **; p<0,01, ***; p<0,001**	**Significant difference in relative anthocyanin con. *; p<0,05, **; p<0,01, ***; p<0,001**
WT WS*35S cpc	n.s.	n.s.
WT WS*cpc-1	*	*
WT COL*try	n.s.	n.s.
WT COL*mybl2	n.s.	n.s.

3.3 Gene expression - seedling stage

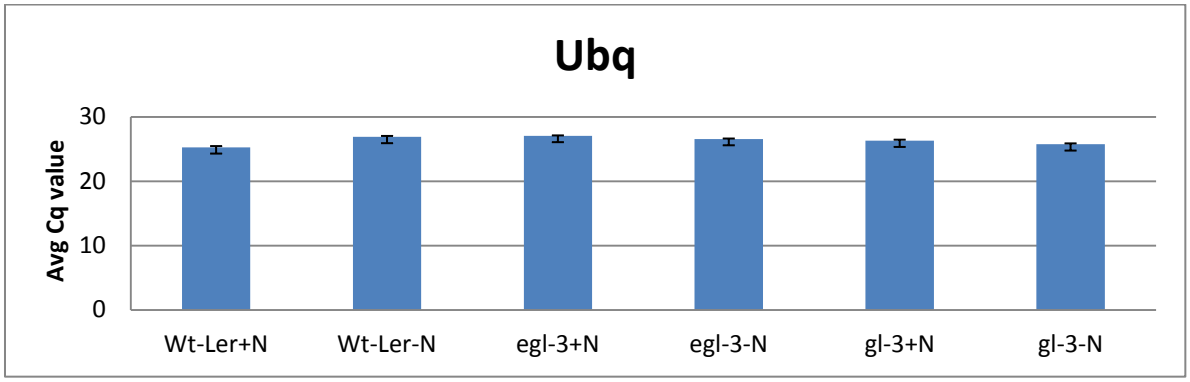
3.3.1 WT Ler, *egl3* and *gl3*

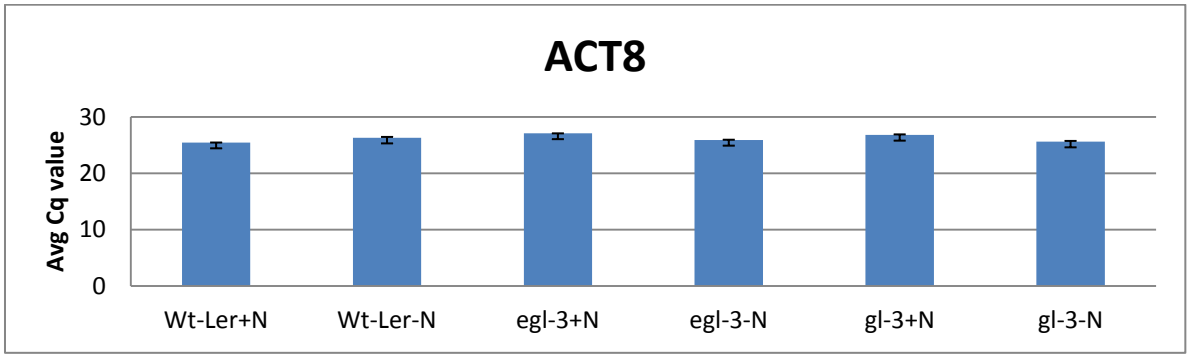
Ler, *egl3* and *gl3* were grown on Petri dishes and treated with and without nitrogen for 7 days in continuous light. Wt and *egl3* plants deprived of nitrogen were small and the leaves were mostly red indicating anthocyanin accumulation. The plants of *gl3* mutant were small and the leaves were yellow, signifying no anthocyanin accumulation. Plants grown with full nitrogen were bigger than those deprived of nitrogen. They were mostly green and did not show much sign of anthocyanin accumulation.

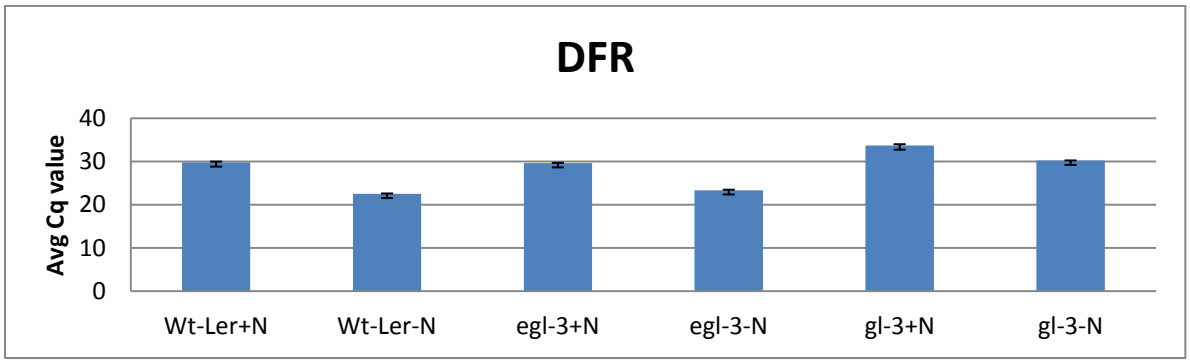
RNA was isolated and the quality measured. A good concentration of RNA could be seen on the agarose gel (Fig. 11) and by Nanodrop (Table A10 in Appendix A).

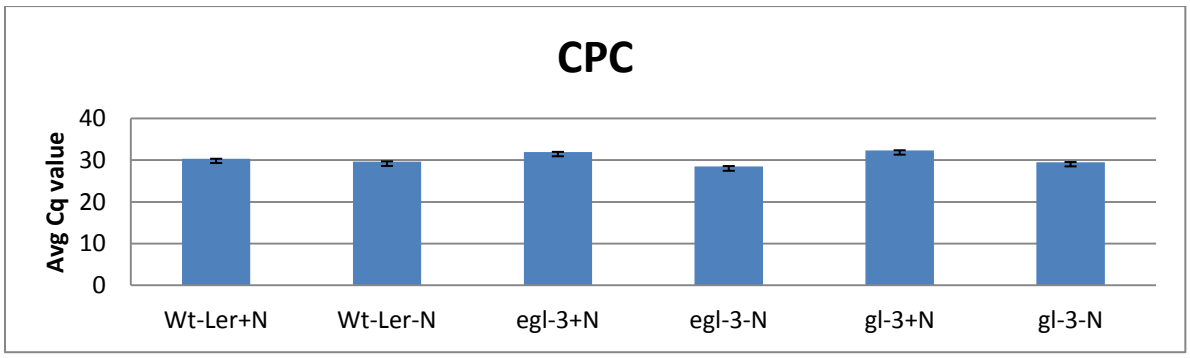
Figure 11. RNA samples run on 1% TAE agarose gel. Line 1) Standard; 2) Wt-Ler +N; 3) *egl3* +N; 4) *gl3* +N; 5) Wt-Ler –N; 6) *egl3* –N and 7) *gl3* –N. Wt-Ler had the lowest concentration. The bonds could be seen more clearly in the original photo.

The gene expression was measured in real time PCR and a correlation between *DFR*, *CPC, MYBL2* and *TRY* was calculated (Fig. 12 – 14). Cq values and RQ values are registered in Tables A11 and A12 in Appendix A. Data presented are means of three replicates from one sample. The differences in gene expression between wild type and the mutants were difficult to interpret. The results were therefore used mainly to look at correlation between the genes and their expression.


Ubq
Avg Cq value
30
20
10
0
Wt-Ler+N
Wt-Ler-N
egl-3+N
egl-3-N
gl-3+N
gl-3-N


ACT8
Avg Cq value
30
20
10
0
Wt-Ler+N
Wt-Ler-N
egl-3+N
egl-3-N
gl-3+N
gl-3-N


DFR
Avg Cq value
40
30
20
10
0
Wt-Ler+N
Wt-Ler-N
egl-3+N
egl-3-N
gl-3+N
gl-3-N


CPC
Avg Cq value
40
30
20
10
0
Wt-Ler+N
Wt-Ler-N
egl-3+N
egl-3-N
gl-3+N
gl-3-N

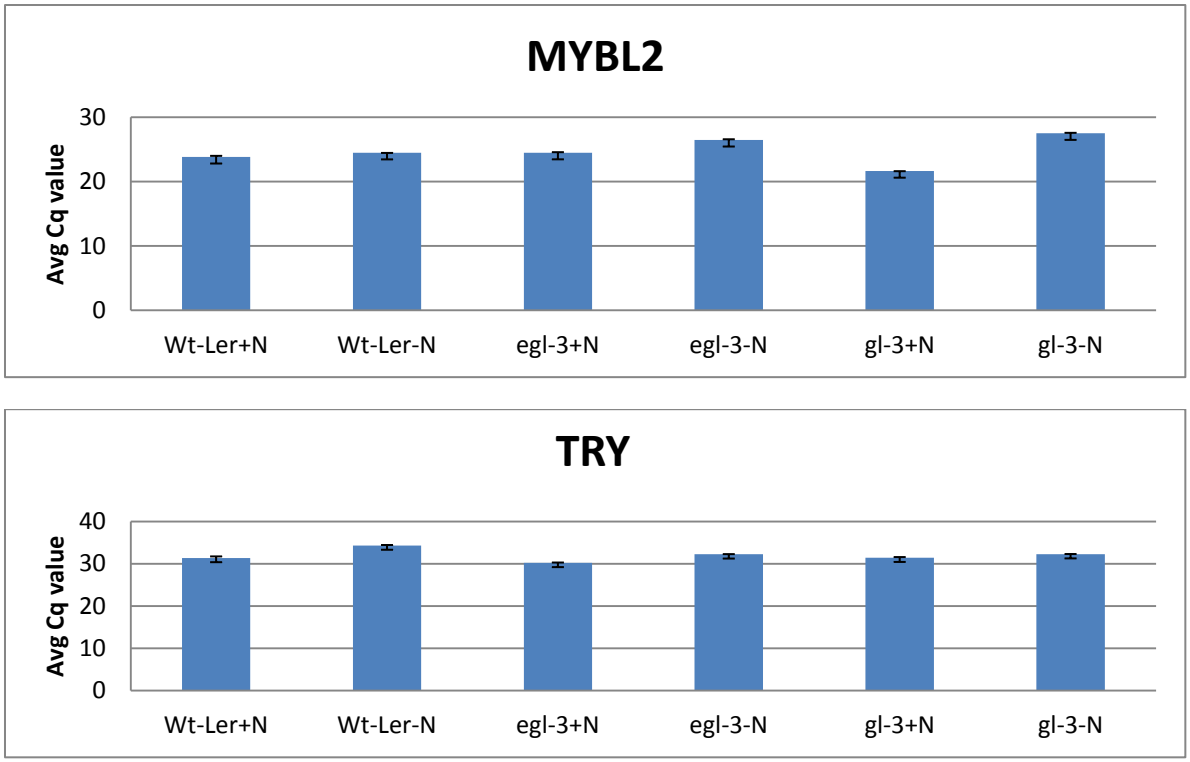


Figure 12. Gene expression. Average Cq values for the genes *DFR*, *CPC*, *MYBL2* and *TRY* in WT-Ler, *egl3* and *gl3*. Plants were grown on Petri dishes and treated with and without nitrogen for 7 days in continuous light. Data presented are means of three replicates from one sample, with standard error.

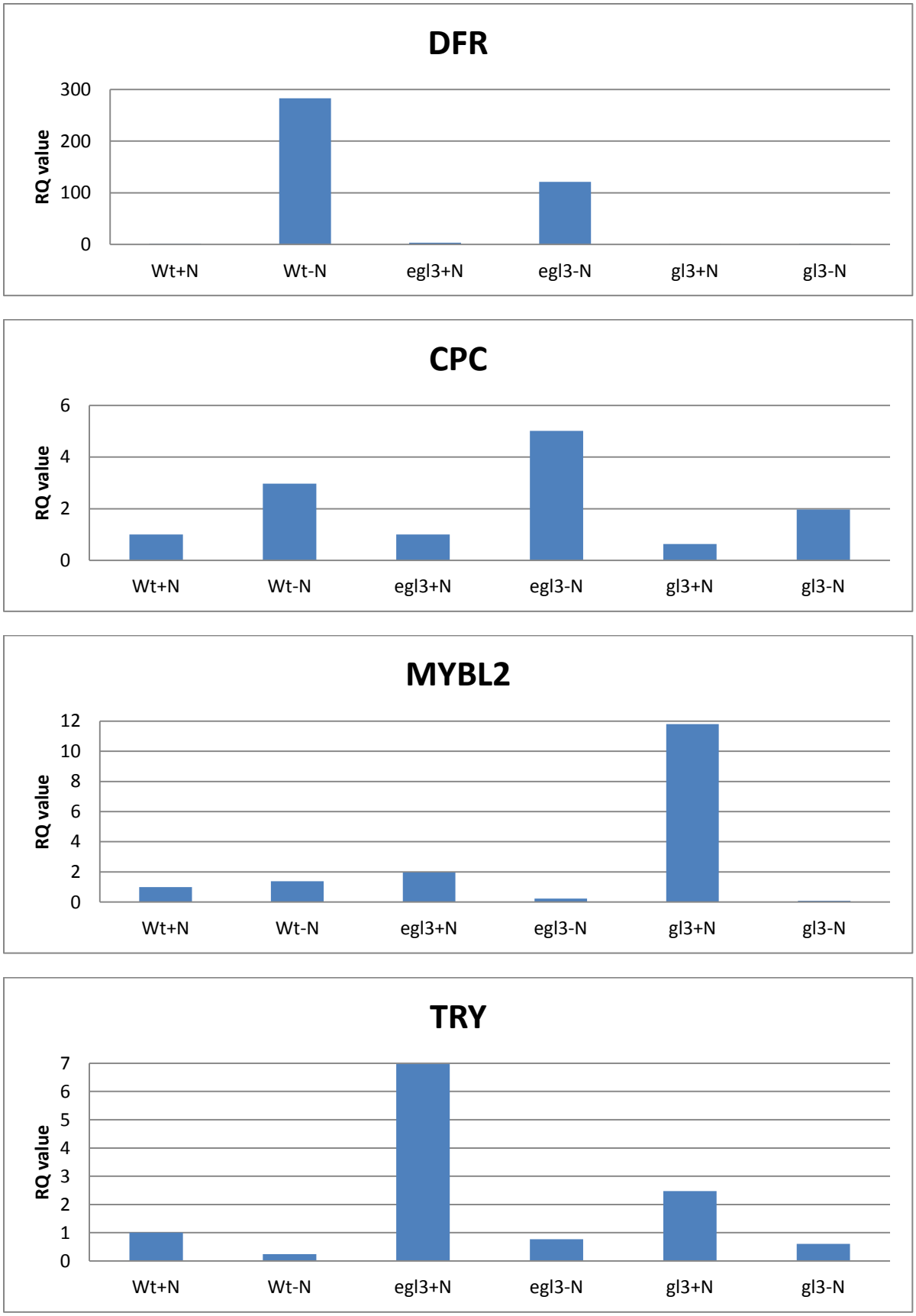
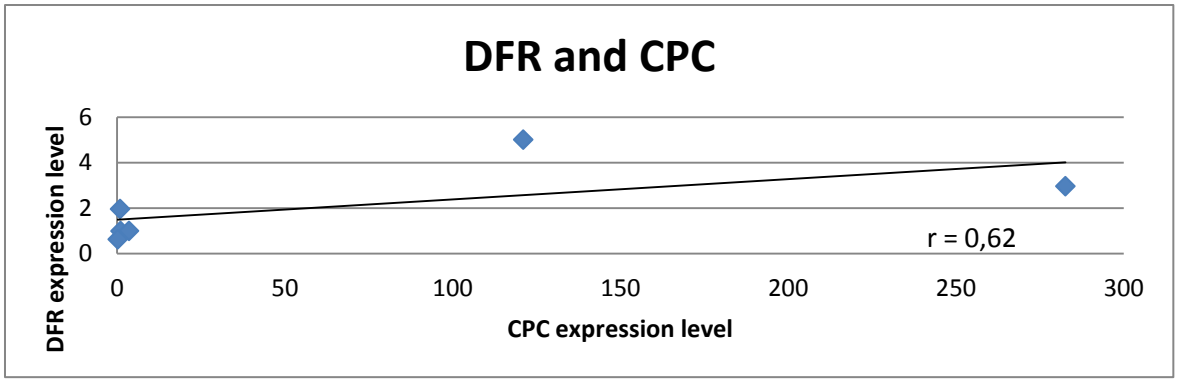
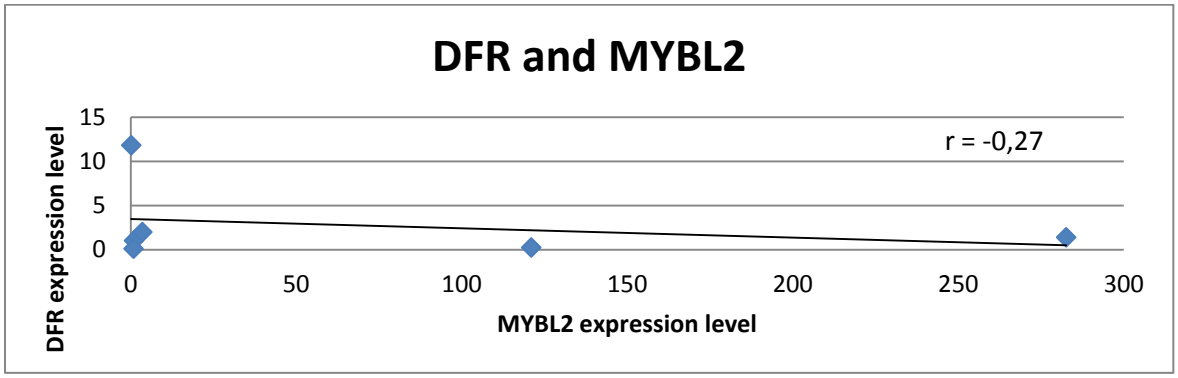
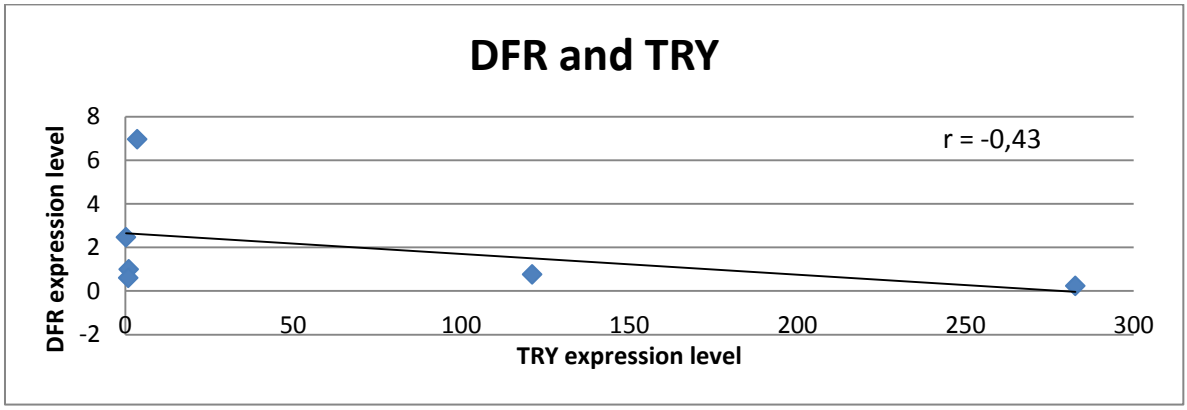


Figure 13. RQ values for the genes *DFR*, *CPC*, *MYBL2* and *Try* in WT-Ler, *egl3* and *gl3*. Plants were grown on Petri dishes and treated with and without nitrogen for 7 days in continuous light. Data presented are means of three replicates from one sample.







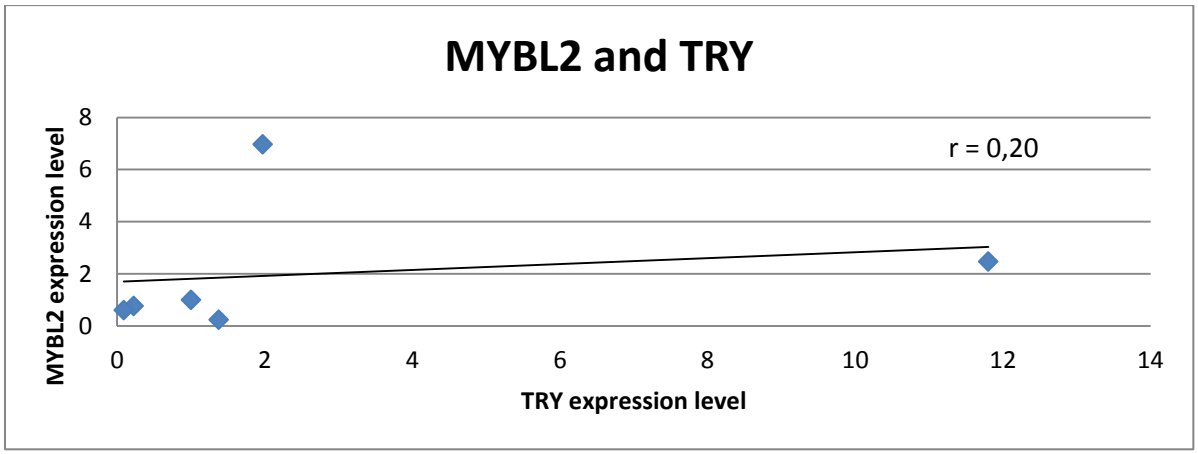


Figure 14. Correlation between expression levels of *DFR* and *CPC; DFR* and *MYBL2*; *DFR* and *TRY*; *MYBL2* and *TRY*. Plants were grown on Petri dishes and treated with and without nitrogen for 7 days in continuous light. Correlation coefficients (r) are shown in the graphs. Data presented are means of three replicates from one sample.

RQ values showed the same trend for *DFR* and *CPC*, where the gene expression went up when the plants were deprived of nitrogen. *gl3* was an exception with inconclusive RQ values. The highest concentration for *DFR* was in WT – N while the strongest gene expression for *CPC* was in *egl*$_3$ – N (Fig. 13 and table A12 in Appendix A).

Reverse trend was seen in gene expression for *MYBL2* and *TRY*. Here, the expression was higher in plants grown with nitrogen. The highest RQ value for *MYBL2* was found in *gl*$_3$ + N while the strongest expression of *TRY* was found in *egl*$_3$ + N (Fig. 13 and Table A12 in Appendix A).

Positive correlation was found between *DFR* and *CPC* (r = 0.62) and *MYBL2* and *TRY* (r = 0.20) (Fig. 14).

3.4 Gene expression – Rosette stage

3.4.1 WT Ler, *egl*$_3$ and *gl*$_3$

WT Ler, *egl*$_3$ and *gl*$_3$ were grown on rock wool and treated for 3 days and 5 days in 16 h light/8 h dark rhythm.

The plants grown with full Hoagland solution were green and little or no anthocyanin accumulation could be seen. Plants that were deprived of nitrogen were darker because of anthocyanin accumulation.

A good RNA concentration was found on the agarose gel (Fig. 15) and by Nanodrop (Table A13 in Appendix A).

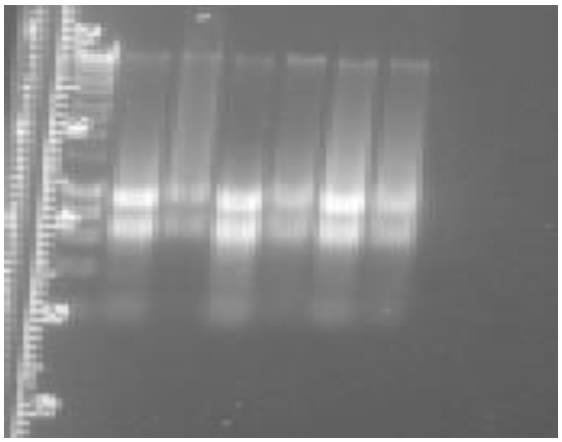
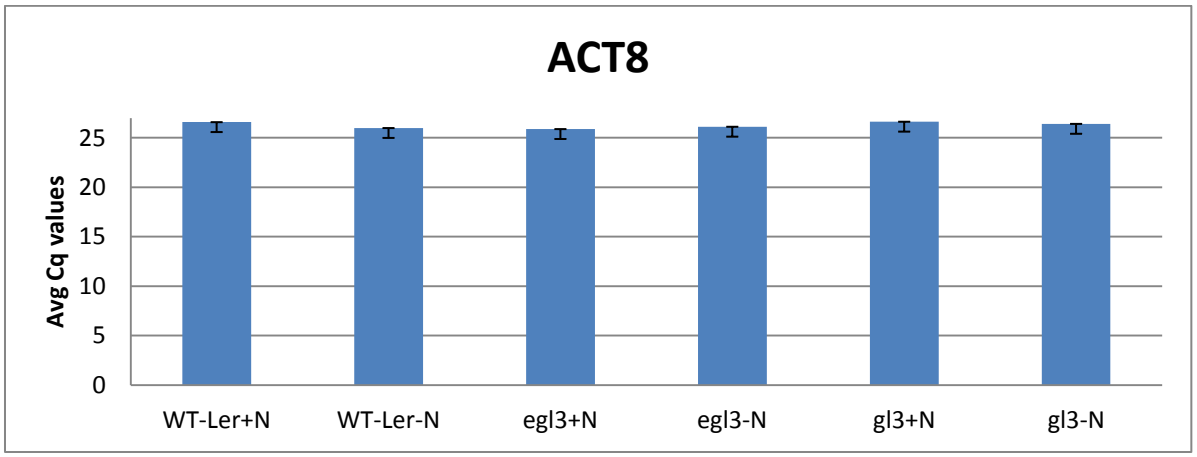
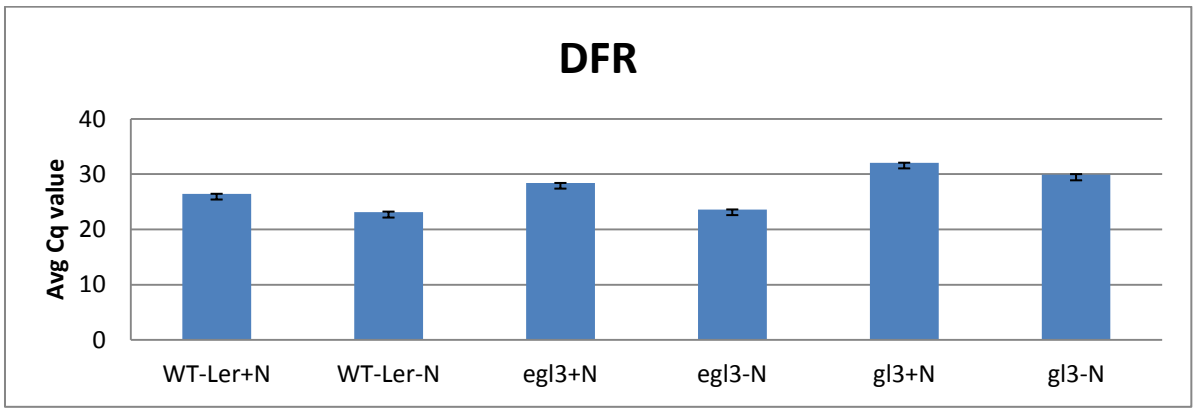
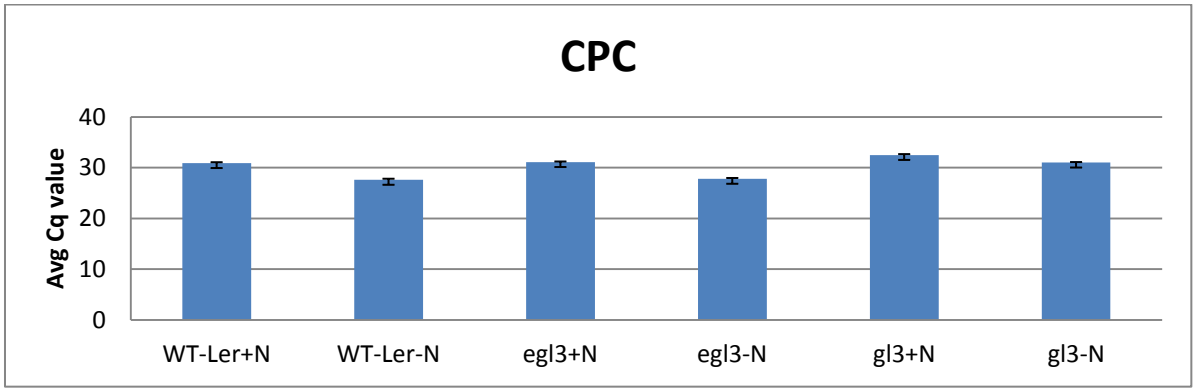


Figure 15. RNA samples run on 1% TAE agarose gel. Line 1) Standard 2) Wt-Ler +N 3) *egl3* +N 4) *gl3* +N and 5) Wt-Ler –N 6) *egl3* –N 7) *gl3* –N. Plants were treated for 3 days in 16 h light/8 h dark rhythm.

The gene expression was measured in real time PCR (Fig. 16 – 18).). Cq values and RQ values are registered in Tables A14 and A15 in Appendix A. Data presented are means of three replicates from one sample. As for the seedling stage, the differences in gene expression between wild type and the mutants were difficult to interpret. The results were therefore used mainly to look at correlation between the genes and their expression.







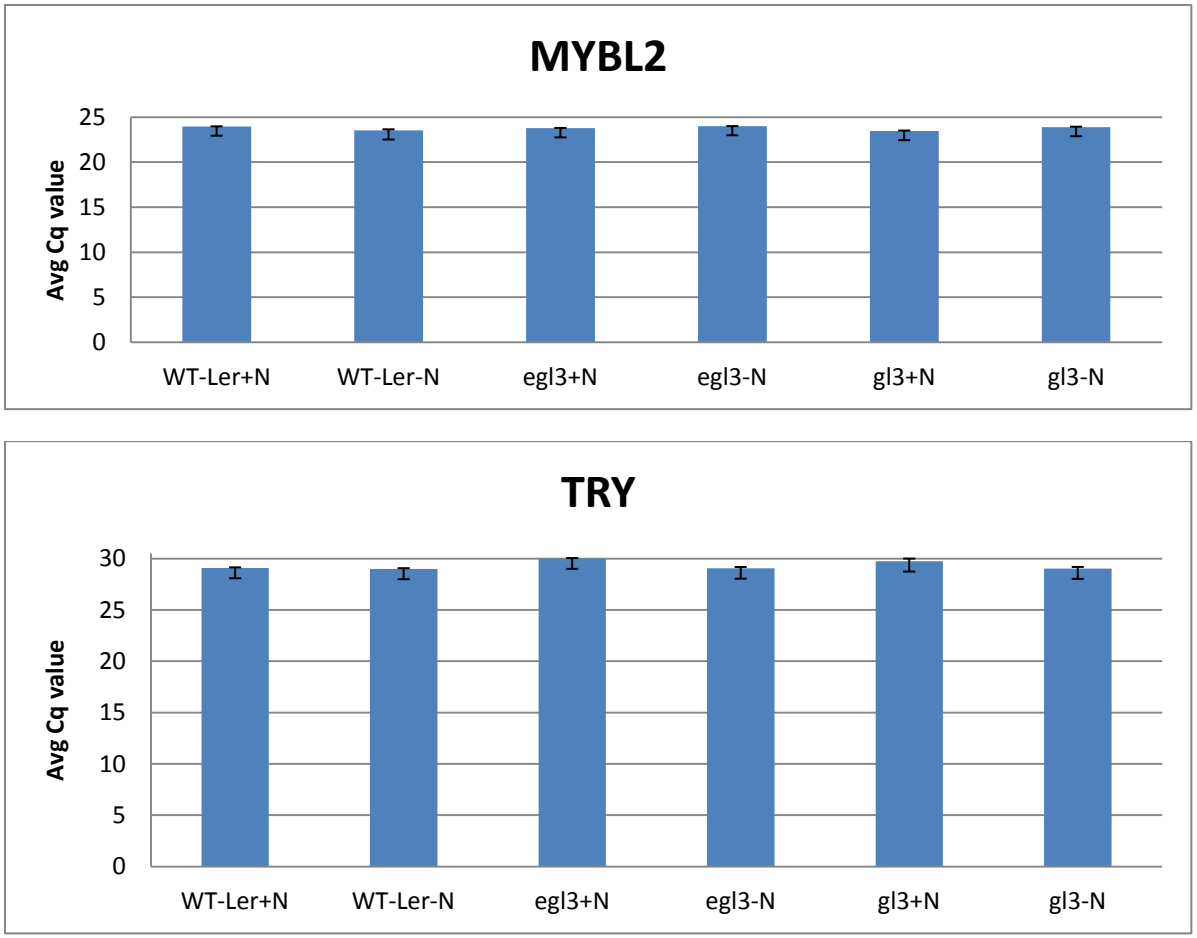


Figure 16. Gene expression. Average Cq values for the genes *ACT8, DFR, CPC, MYBL2 and TRY* in WT-Ler, *egl3* and *gl3*. Plants were grown on rock wool and treated with and without nitrogen for 3 days in 16 h day/8 h night rhythm. Data presented are means of three replicates from one sample, with standard error.

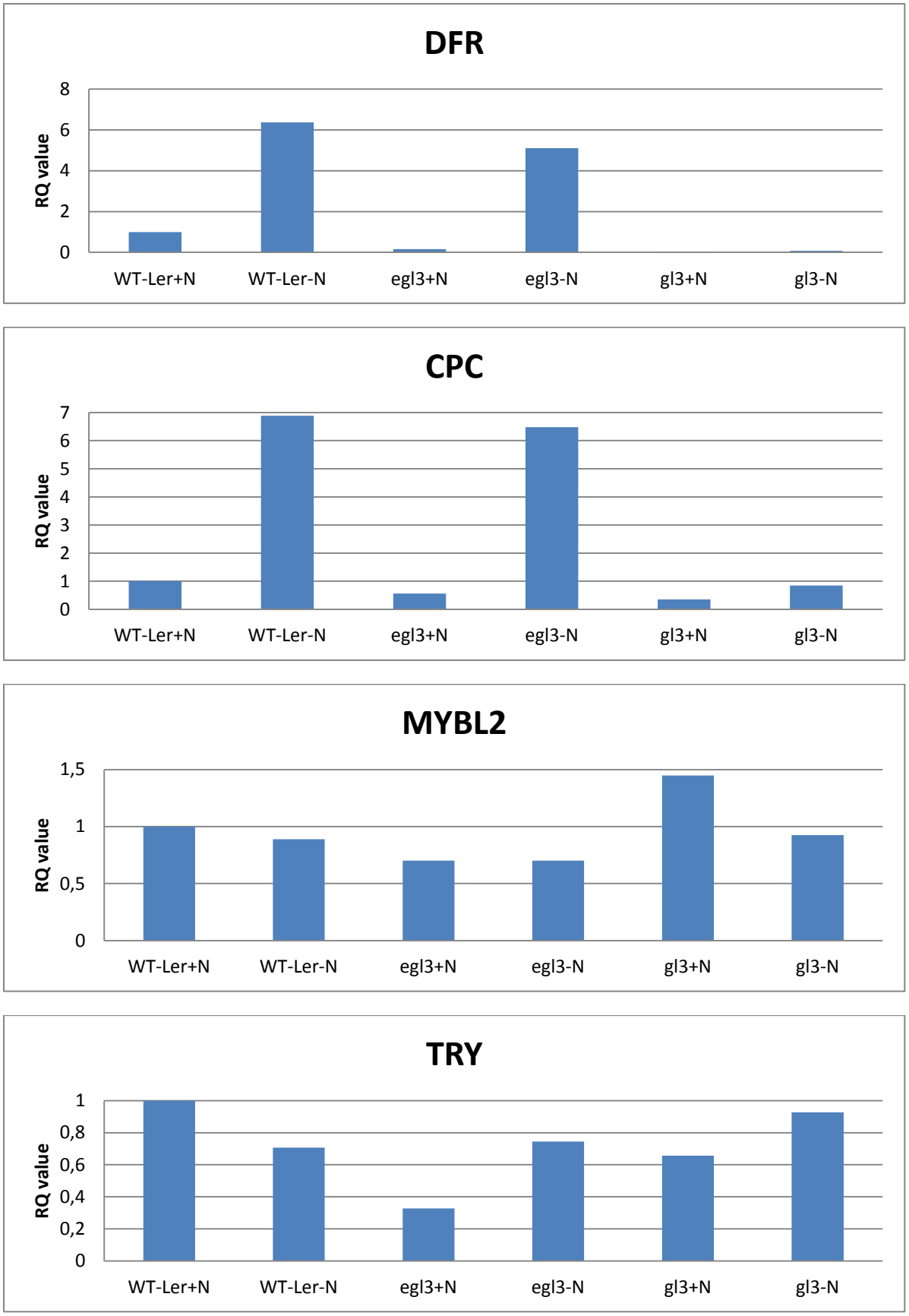


Figure 17. RQ values for the genes *DFR, CPC, MYBL2* and *TRY* in WT-Ler, egl3 and gl3. Plants were grown on rock wool and treated with and without nitrogen for 3 days in 16h day/8h night rhythm. Data presented are means of three replicates from one sample.

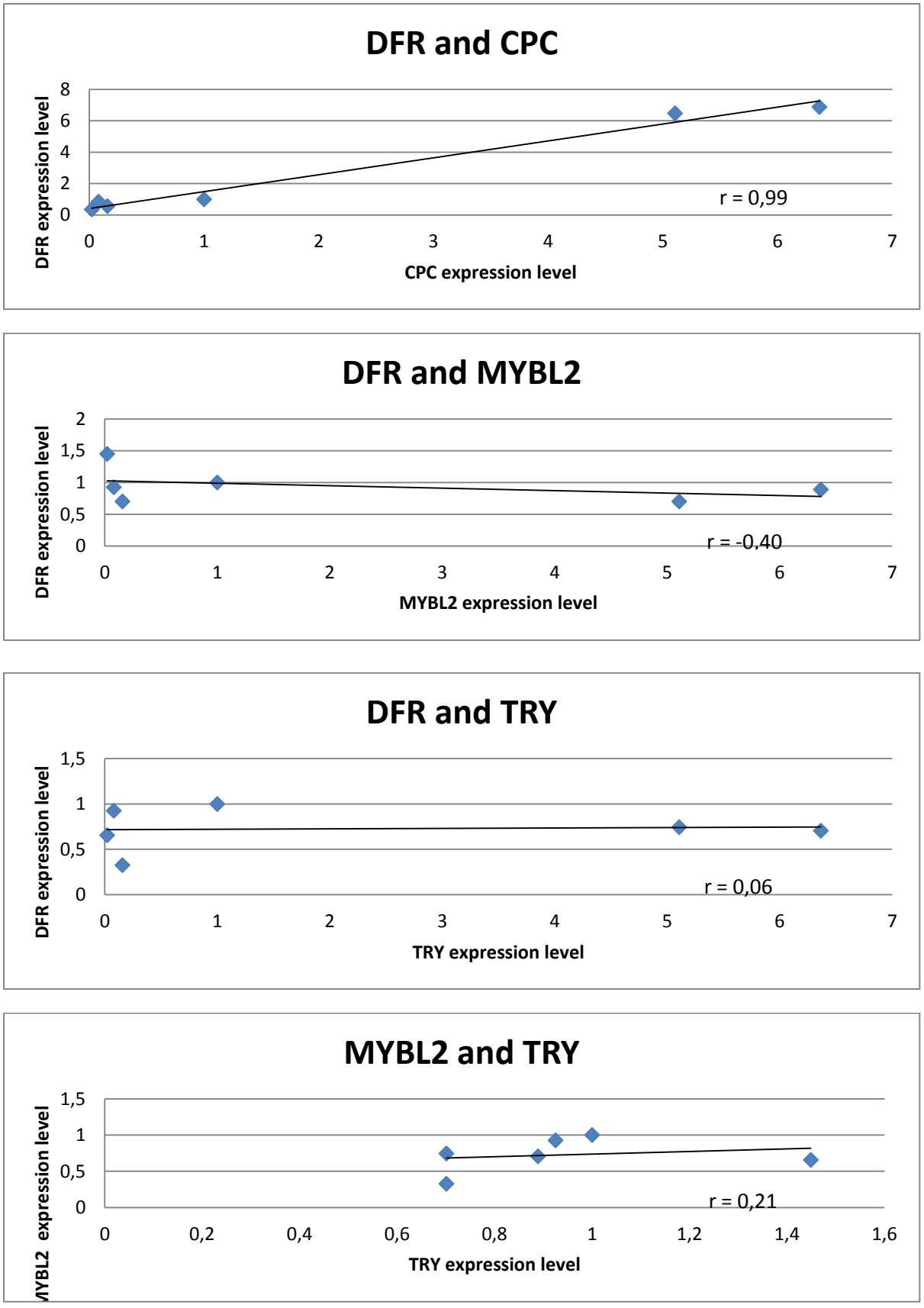
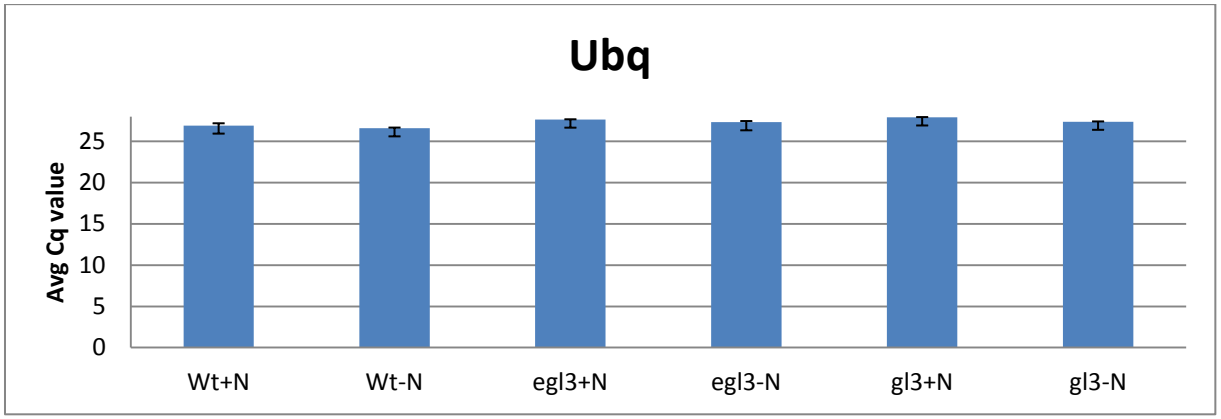


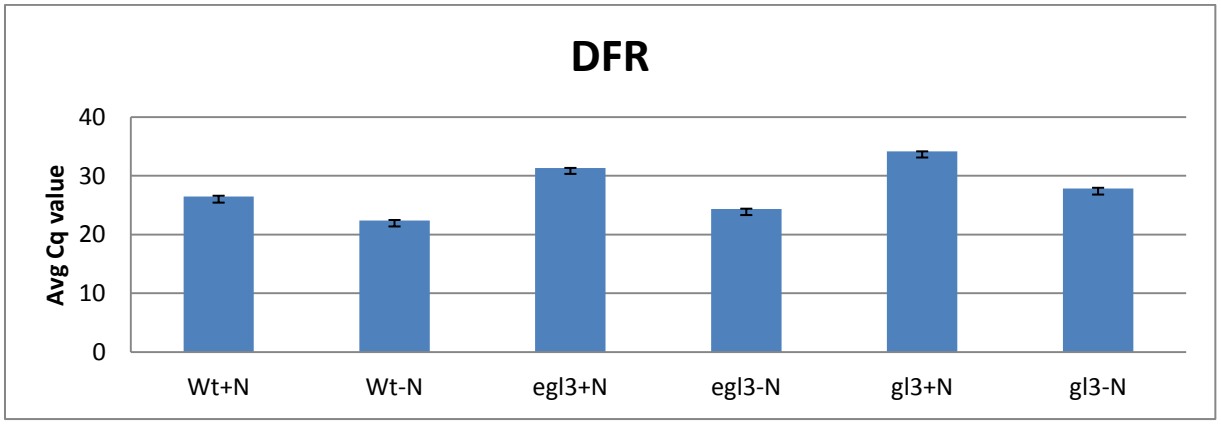
Figure 18. Correlation between expression levels of *DFR* and *CPC, DFR* and *MYBL2, DFR* and *TRY, MYBL2* and *TRY.* Plants were grown on rock wool and treated with and without nitrogen for 3 days in 16h day/8h night rhythm. Correlation coefficients (r) are shown in the graphs.

The same trend was seen in *DFR* and *CPC* where the RQ values were higher in plants treated without nitrogen. The same pattern could also be seen in the expression of *MYBL2* and *TRY* in wild type while the contrast was seen in the mutants. It must be noted that in WT+N *TRY,* only one replicate was used and this could have affected the results (Fig. 17).

There was a strong positive correlation between *DFR* and *CPC* (r = 0.99). There was also found a relationship between *MYBL2* and *TRY* and a weak correlation between *DFR* and *TRY (*r=0.06) (Fig. 18).

The gene expression was also measured in plants grown on rock wool and treated with and without nitrogen for 5 days (Fig. 19 – 21). Cq values and RQ values are found in tables A16 and A17 in Appendix A. Data presented are means of three replicates from one sample.





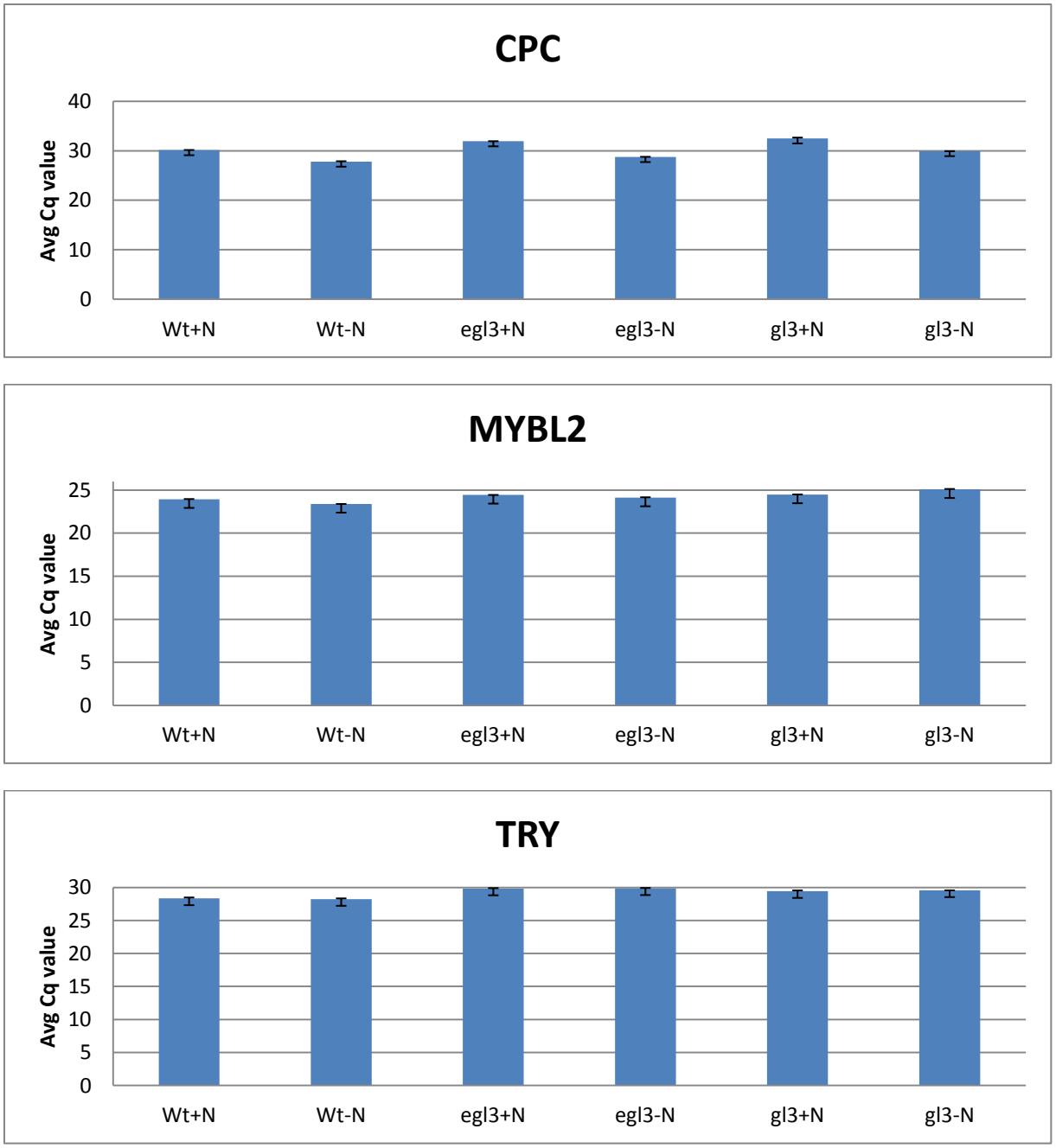


Figure 19. Rosette stage. Average Cq values for the genes *DFR, CPC, MYBL2* and *TRY* in WT-Ler, *egl3* and *gl3*. Plants were grown on rock wool and treated with and without nitrogen for 5 days in 16h day/8 h night rhythm. Data presented are means of three replicates from one sample, with standard error.

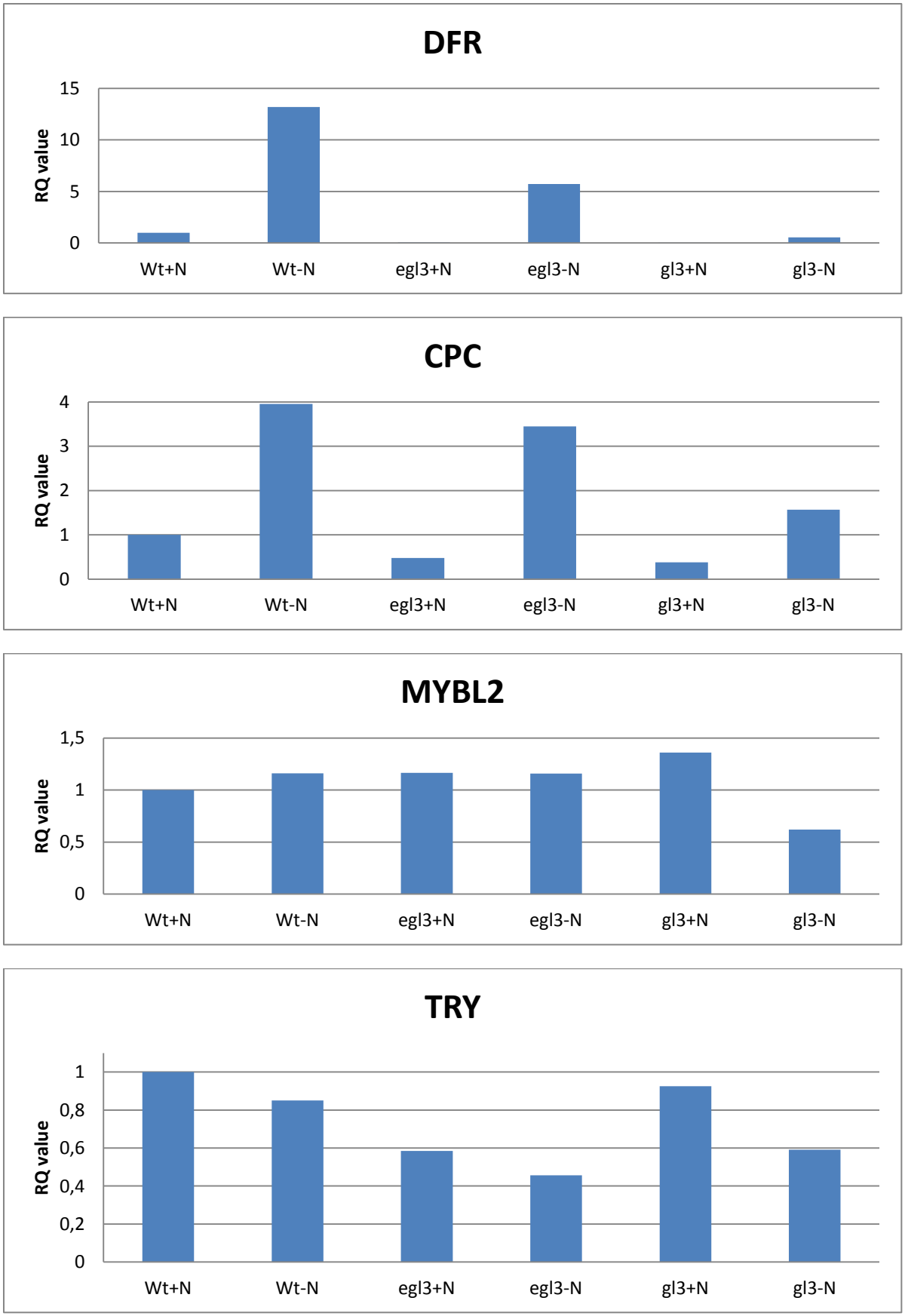
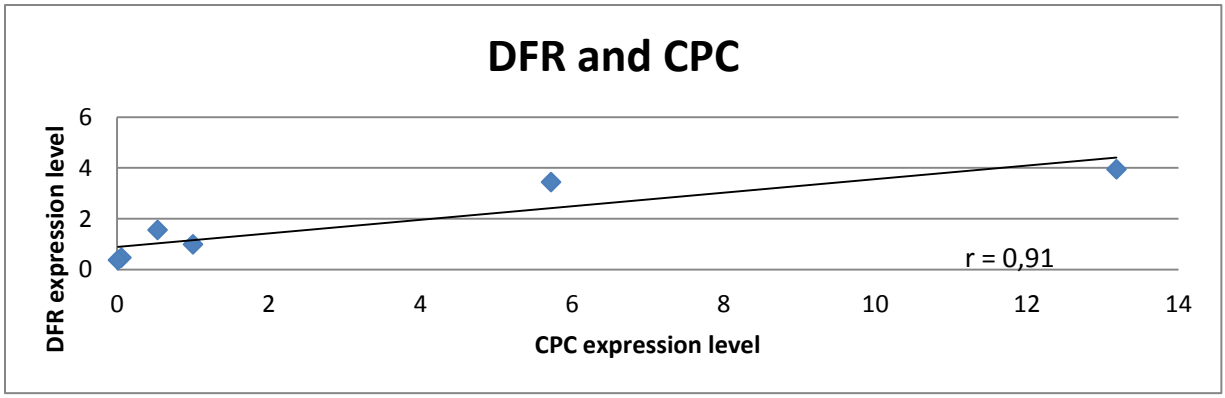
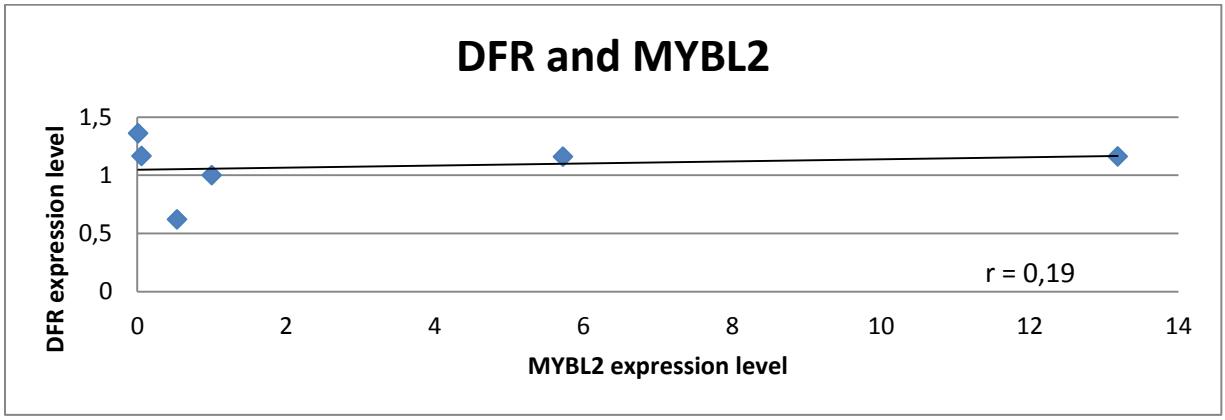
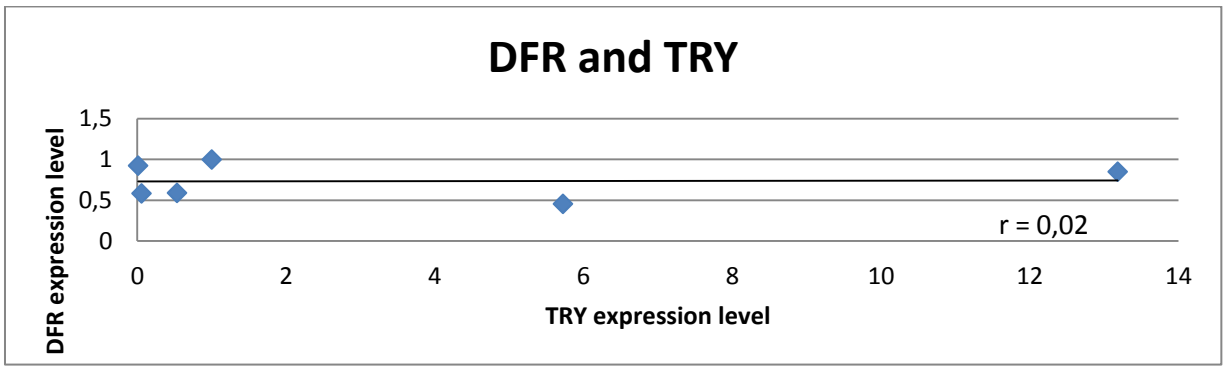


Figure 20. Rosette stage. RQ values for the genes *DFR, CPC, MYBL2* and *Try* in WT-Ler, *egl3* and *gl3*. Plants were grown on rock wool and treated with and without nitrogen for 5 days in 16h day/8 h night rhythm. Data presented are means of three replicates from one sample.







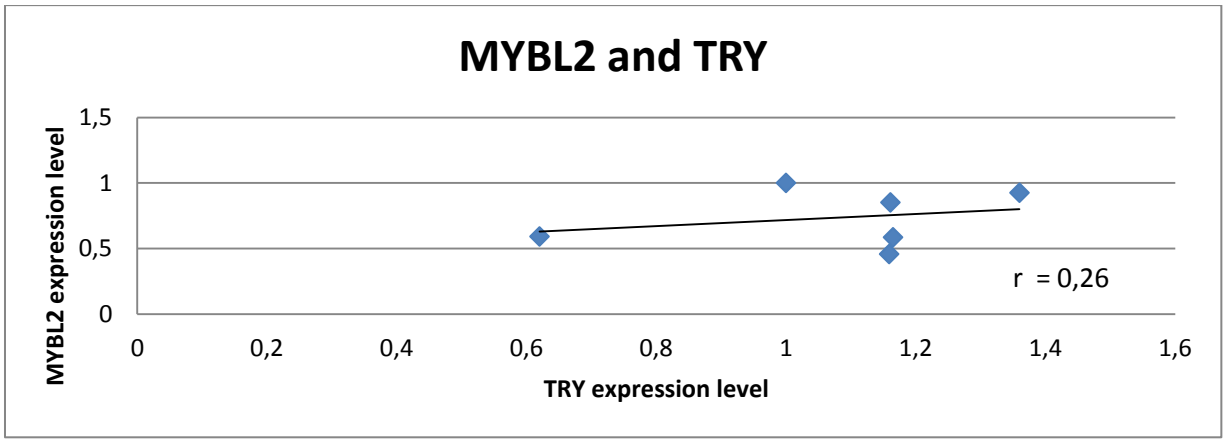


Figure 21. Correlation between expression levels of *DFR* and *CPC, DFR* and *MYBL2, DFR* and *TRY, MYBL2* and *TRY*. Plants were grown on rock wool and treated with and without nitrogen for 5 days in 16h day/8 h night rhythm. Correlation coefficients (r) are shown in the graphs.

The same trend could be seen in *DFR* and *CPC*, where the gene expression went up in plants deprived of nitrogen. RQ values in *TRY* show the same pattern as in plants harvested at seedling stage. In M*YBL2* RQ values are similar in wild type and egl_3 grown with and without nitrogen. In gl_3, the expression of *MYBL2* went clearly down (Fig. 20).

A strong positive correlation was found between *DFR* and *CPC* (r = 0.91). The correlation for the other genes was weaker (Fig. 21).

3.4.2 WT WS and WT Col

Plants were grown on rock wool and treated with and without nitrogen for 5 days in continuous light and 16h day/8 h night rhythm. Plants grown with full Hoagland solution were green and no anthocyanin accumulation could be detected visually. Plants that were deprived of nitrogen were darker because of anthocyanin accumulation.

RNA was isolated from the samples and a good quality could be seen on agarose gel (Fig. 22) and by Nanodrop (Tables A18 and A19 in Appendix A).

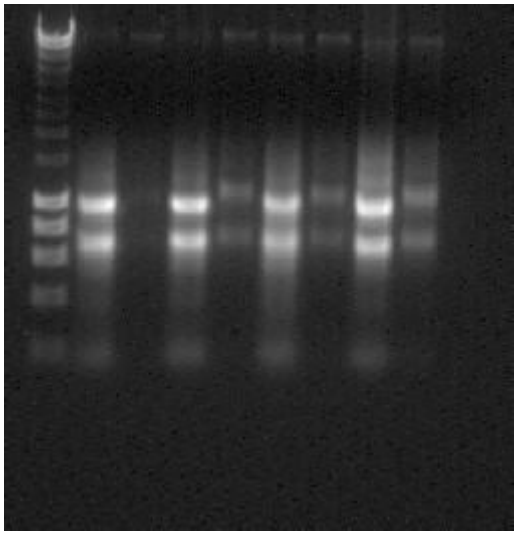
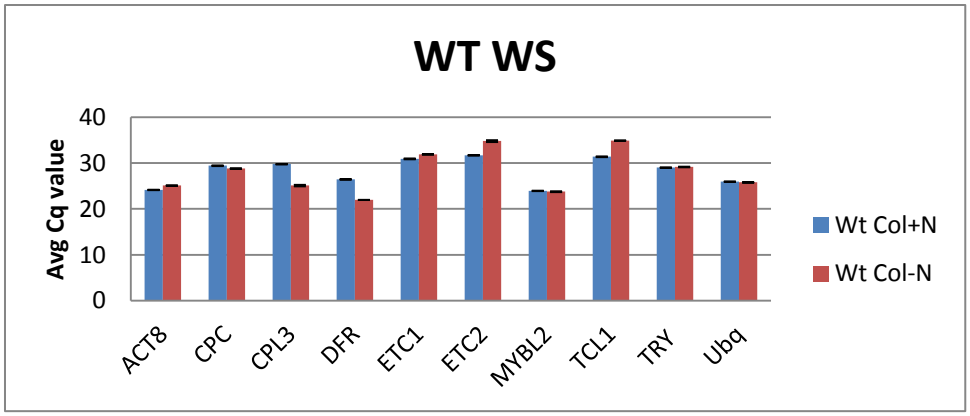


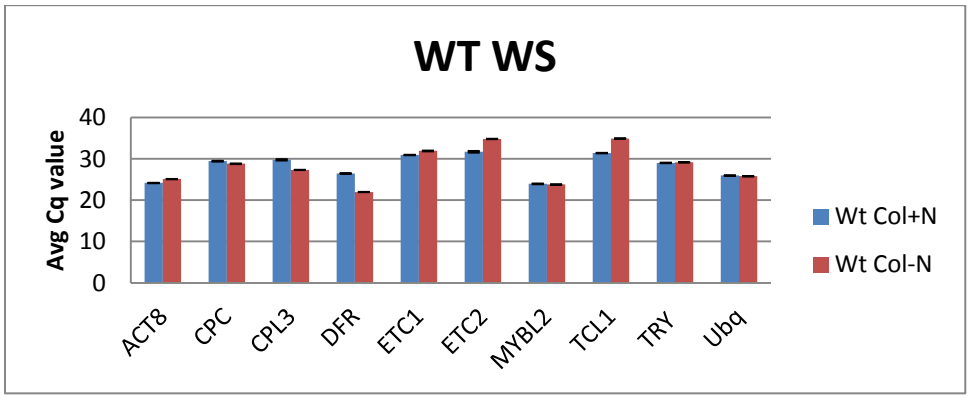
Figure 22. RNA samples run on 1% TAE agarose gel. Line 1) Standard 2) Wt-WS +N continuous light 3) WT WS –N continuous light 4) WT COL +N continuous light 5) Wt-COL –N continuous light 6) WT WS +N 16 h light/8 h dark rhythm 7) WT WS –N 16 h light/8 h dark rhythm 8) WT COL +N 16 h light/8 h dark rhythm 9) WT COL -N 16 h light/8 h dark rhythm. The bonds could be seen more clearly in the original photo.

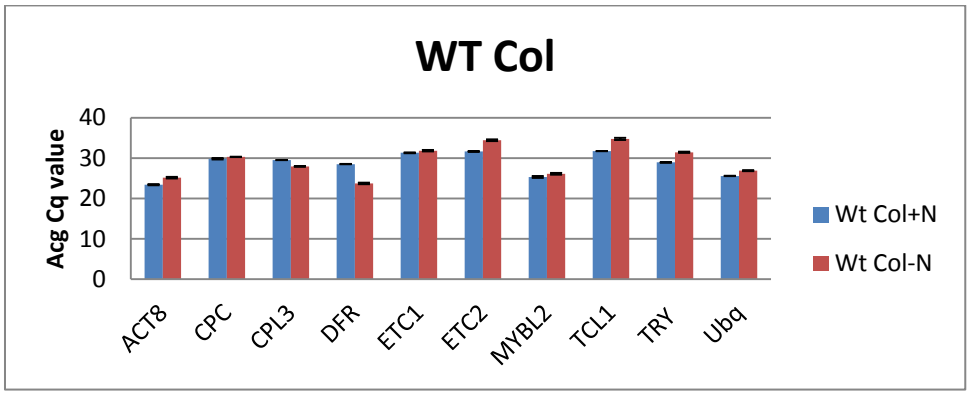
Continuous light

Plants were grown in continuous light and treated with and without nitrogen for 5 days. The gene expression of the plants was measured in real time PCR (Fig. 23 and 24). Cq and RQ values can be found in Tables A20 and A21 in Appendix A.

Figure 24 illustrates gene expression in WT WS and WT Col grown in continuous light. There was a difference in the expression when different endogenous controls were used, *Ubq* giving stronger expression. The trend is similar in both wild types except for WT WS when *ACT8* is used, where *ETC1* and *TRY* show opposite expression. *CPC, CPL3, ETC1* and *MYBL2* showed the same trend as *DFR*, while *ETC2, TCL1* and *TRY* showed the opposite tendency.







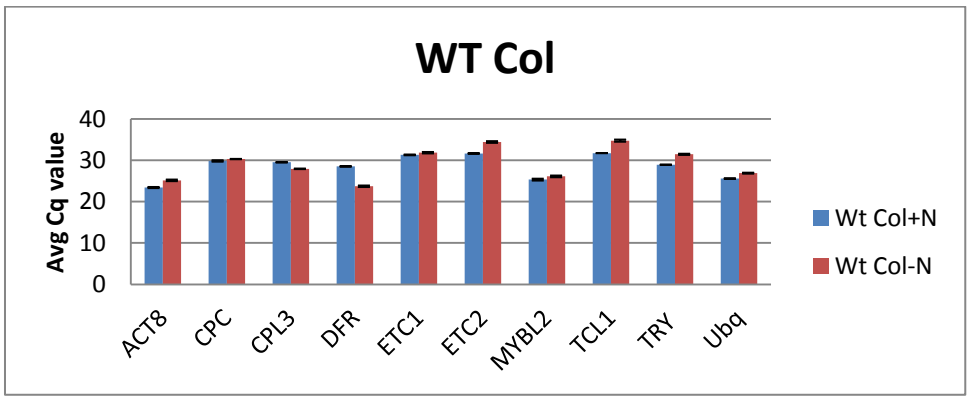
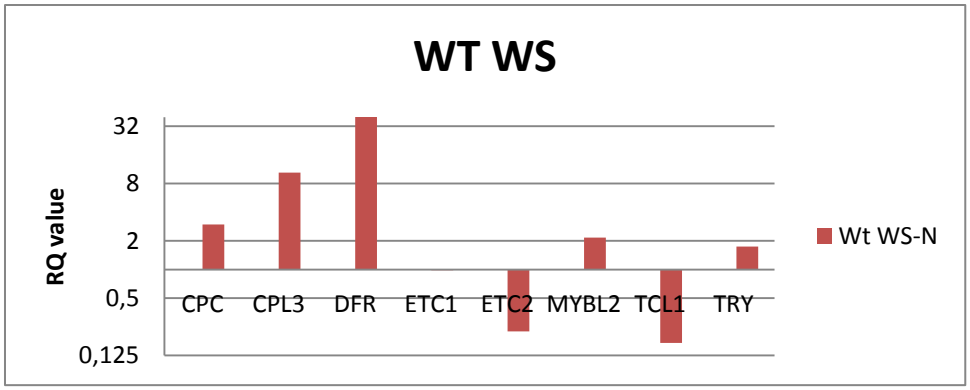
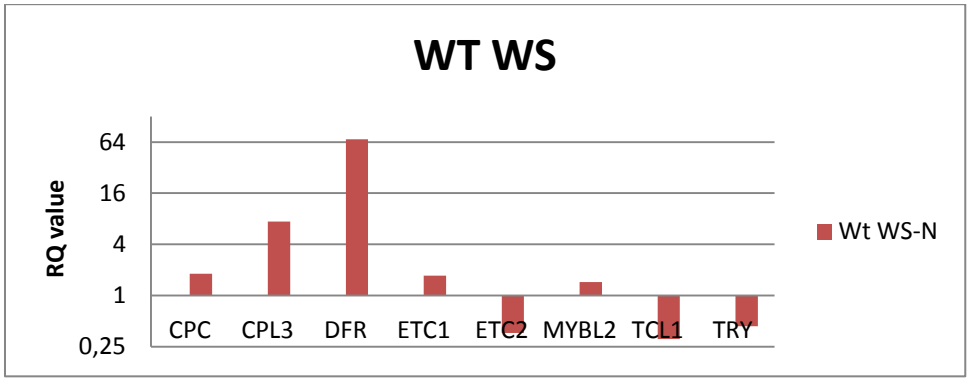
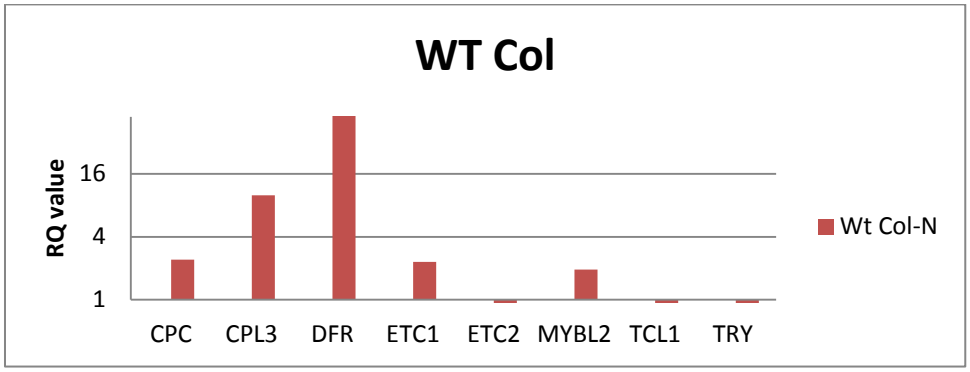


Figure 23. Rosette stage. Average Cq values for the genes *CPL3, CPC, DFR, ETC1, ETC2, MYBL2, TCL1* and *TRY* in WT WS and WT Col. The plants where grown in continuous light and were treated with and without nitrogen for 5 days. In graph 1 and 3 *ACT8* was used as endogenous control. In graph 2 and 4 *Ubq* was used as endogenous control. Data presented are means of three replicates from one sample.







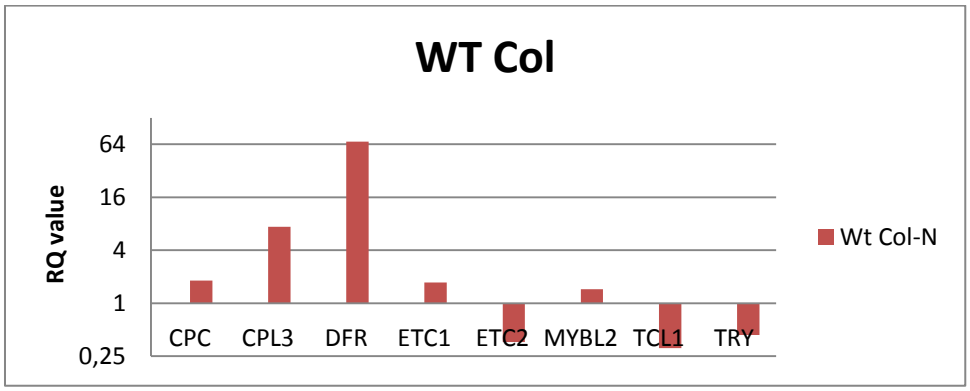
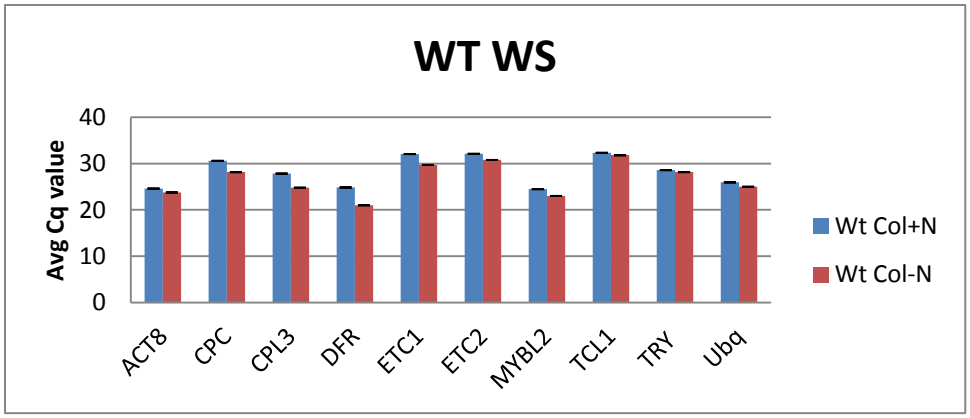


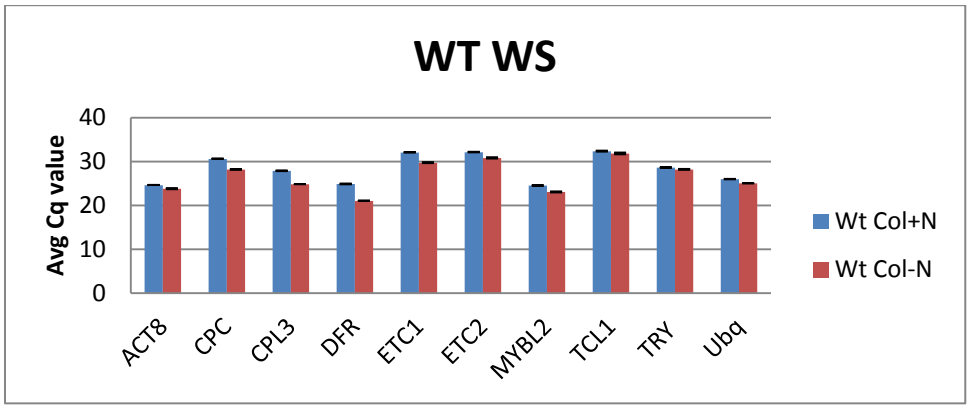
Figure 24. Rosette stage. RQ values for the genes *CPL3, CPC, DFR, ETC1, ETC2, MYBL2, TCL1* and *TRY* in WT WS and WT col. The plants where grown in continuous light and were treated with and without nitrogen for 5 days. In graph 1 and 3 *ACT8* was used as endogenous control. In graph 2 and 4 *Ubq* was used as endogenous control. WT + N was used as a calibrator. The y-axis is in logarithmic scale, base 2). Data presented are means of three replicates from one sample.

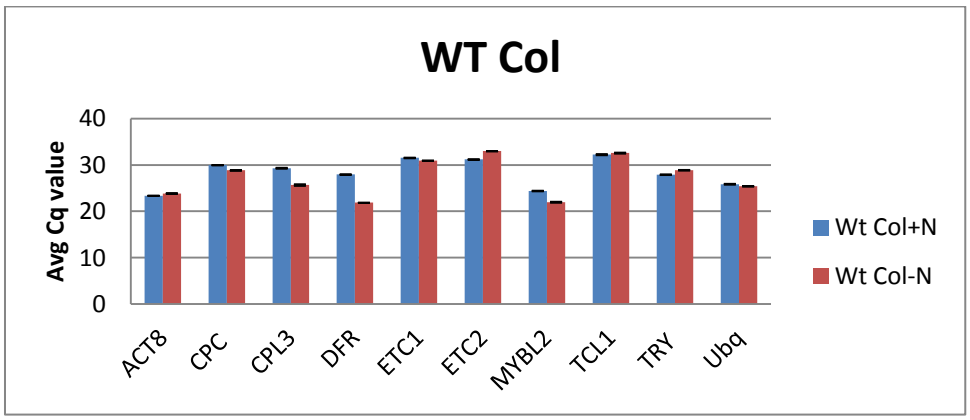
16 h day/8 h night day rhythm

Plants were grown in 16 h day/8 h night rhythm and treated with and without nitrogen for 5 days. The gene expression of the plants was measured in real time PCR (Fig. 25 and 26). Cq and RQ values can be found in Tables A22 and A23 in Appendix A.

For plants grown in 16 h/8 h regime, a clear difference could be seen in gene expression for WT WS and WT Col, where the expression was stronger in WT Col. When WT WS was deprived of nitrogen, the expression of all genes went up except for *TCL1* and *TRY* where the gene expression went down. The same trend was seen for WT Col except for *ETC2* where the gene expression went down when the plants did not receive nitrogen.







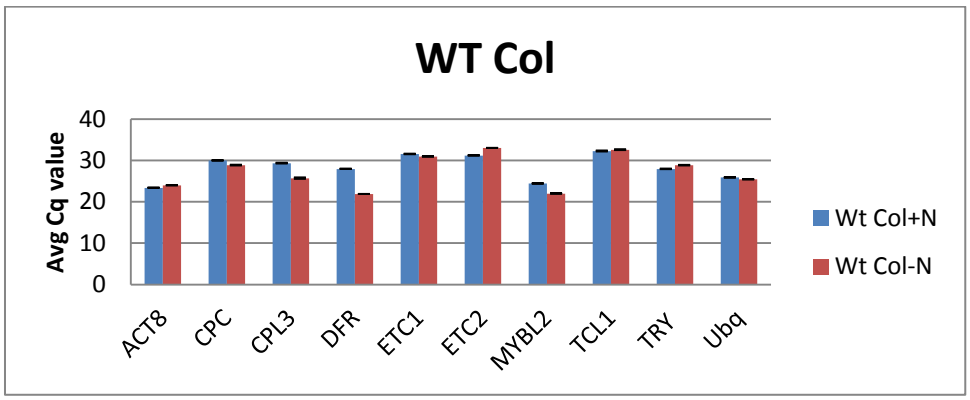


Figure 25. Rosette stage. Average Cq values for the genes *CPL3, CPC, DFR, ETC1, ETC2, MYBL2, TCL1* and *TRY* in WT WS and WT Col. The plants where grown in 16 h/8 h day rhythm and were treated with and without nitrogen for 7 days. In graph 1 and 3 *ACT8* was used as endogenous control. In graph 2 and 4 *Ubq* was used as endogenous control. Data presented are means of three replicates from one sample.

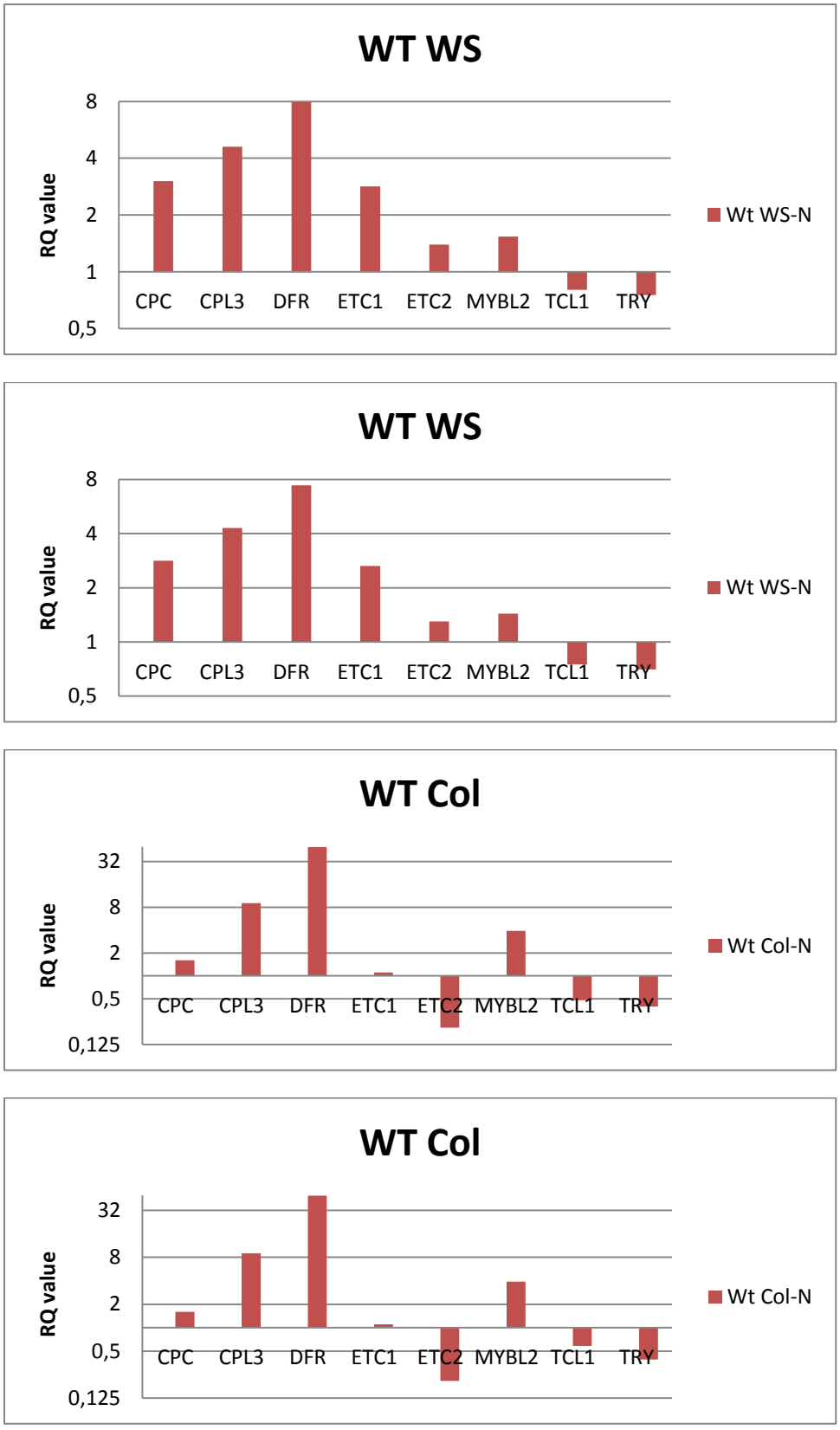


Figure 26. Rosette stage. Average RQ values for the genes *CPL3, CPC, DFR, ETC1, ETC2, MYBL2, TCL1* and *TRY* in WT WS and WT Col. The plants where grown in 16 h/8 h day rhythm and were treated with and without nitrogen for 7 days. In graph 1 and 3 *ACT8* was used as endogenous control. In graph 2and 4 *Ubq* was used as endogenous control and WT + N was used as a calibrator. The y-axis is in logarithmic scale, base 2). Data presented are means of three replicates from one sample.

For plants grown in 16 h/8 h regime, a clear difference could be seen in gene expression for WT WS and WT Col, where the expression was stronger in WT Col. When WT WS were deprived of nitrogen, the expression of all genes went up except for *TCL1* and *TRY* where the gene expression went down. The same trend was seen for WT Col except for *ETC2* where the gene expression went down when the plants did not receive nitrogen.

4 Discussion

In anthocyanin measuring of WT WS overexpression line *35 cpc* and the knockout mutant *cpc-1*, the concentration in WT WS and *35S cpc* was found to be similar. The mutant *cpc-1* accumulated more anthocyanins than the other ecotypes and a significant difference between wild type and this mutant was found when the plants were grown in 16 h day/8 h light rhythm. Zhu and coworkers (2009) found CPC to be a negative regulator of anthocyanins where the *35S cpc* plants accumulated less antohcyanins than WT WS, and *cpc-1* mutants had higher anthocyanins concentration than wild type. In this experiment, the same trend could only be seen between wild type and *cpc-1*. The reason for the different outcomes could be that in the work of Zhu, the plants were stressed with very high light intensity but the flux used in this experiment was lower and resembled more what is found in nature. It must be noted that in measurement 2 (continuous light, see appendix A), *35S cpc* mutant accumulated less anthocyanins than wild type supporting the findings of Zhu and coworkers.

The mutant *mybl2* accumulated more anthocyanins than WT Col in continuous light and 16 h/8 h day rhythm, the difference being higher in continuous light. *try* had similar concentration as the wild type but produced more anthocyanins when grown in 16h day/8 h night rhythm. *mybl2* has been found to be an inhibitor in anthocyanin synthesis (Dubos et al. 2008; Matsui et al. 2008) and these results show the same trend when the plants were grown in continuous light. MYBL2 might therefore be an inhibitor in anthocyanin synthesis, especially when high light factors influence the plants. *try* followed mostly the same trend as *mybl2* and could therefore be a possible candidate for being important in anthocyanin synthesis.

The ecotypes WT Ler, *egl3* and *gl3* were grown on Petri dishes and rock wool. There was observed a visual difference in color change in egl_3 and gl_3 where the former were red but gl_3 became yellow. gl_3 accumulated no anthocyanins which indicates its importance in anthocyanin accumulation. This supports the finding of Feyissa and coworkers in 2009.

Analysis of gene expression in WT Ler, *egl3* and *gl3* showed that *CPC* followed the same trend as *DFR* and there was a strong correlation between these genes. This was unexpected since *CPC* has been found to be a negative regulator of anthocyanins while DFR has been recognized as a catalyst in the same process. This indicates therefore that CPC is not an inhibitor in anthocyanin biosynthesis. A difference was found in the expression of *DFR* in the egl_3 mutant and the gl_3 mutant where *DFR* was expressed more in egl_3. This supports the findings of Feyissa (2009) where GL_3 was found to be more necessary than EGL_3 in anthocyanin accumulation. The same was found in the expression of *CPC*.

The correlation between *MYBL2* and *TRY* was positive but not as exclusively as was seen among *DFR* and *CPC*. Weak or no correlation was found between *MYBL2* and *DFR*. This supports the results found in anthocyanin measuring. The correlation between *TRY* and *MYBL2* was positive in all experiments and the correlation between *TRY* and *DFR* was negative or around zero. TRY might therefore be a candidate as an inhibitor in anthocyanin biosynthesis.
It must be noted that these results are preliminary because they come from only one parallel and it might be necessary to perform more studies to get more conclusive answers.

To get a better understanding of these genes, transcription analysis was made in WT WS and WT Col where other R3 MYB factors were included. *ACT8* and *Ubq* were used as endogenous controls. *Ubq* was expressed more constant than *ACT8* and was therefore a better candidate as a control. A difference in gene expression was noticed when different endogenous were used. This is because gene expression is given relative to the endogenous controls. Higher expression in *Ubq* than in *ACT8* leads to higher gene expression in all genes that are measured relative to *Ubq*.
DFR was strongly expressed in plants deprived of nitrogen which was expected since it is known from earlier researches that DFR catalysis the formation of leucocyanidin and leucoanthocyanidin in anthocyanin biosynthesis. *CPC* followed the same trend as *DFR* but the expression was lower. This is the same pattern as was seen in WT, *egl*$_3$ and *gl*$_3$ giving a stronger indication that CPC is not an inhibitor in anthocyanin biosynthesis. *CPL3* and *ETC1* followed the same pattern as *DFR* and *CPC* and might therefore have a role in anthocyanin synthesis.
It was expected to see the RQ value for *MYBL2* to become lower when the plants were deprived of nitrogen as was seen in the other experiments in this study, but the results here show the contrast. This might indicate that MYBL2 is not an inhibitor in anthocyanin synthesis which is in contrast to what has been found in earlier studies (Dubos et al. 2008 and Matsui et al. 2008).
The expression of *TRY* decreased in both wild types when the plants were grown without nitrogen showing the same results as in WT Ler, *egl*$_3$ and *gl*$_3$. *TCL1* followed the same pattern as *TRY* and might therefore be involved in inhibiting anthocyanin accumulation.
The expression of *ETC1* varied between wild types. It was expressed positively in WT WS but a strong negative expression was seen in WT Col. This might indicate different roles in these two wild types. Growth conditions or a difference in treatment of these two ecotypes might explain different expression of this gene but since all the other genes show the same trend this is an unlikely explanation.
The expression of these R3 MYBL genes is interesting but preliminary so further studies must be made. It should be repeated to see if the same results are gained, especially for MYBL2 which

showed unexpected results. Various mutants could also be included to get a better understanding of interaction and evolvement of these R3 MYBL factors.

5. Conclusion

The study shows that the MYB proteins MYBL2, CPC and TRY are influenced by nitrogen depletion and other stress factors. The results indicate that MYBL2 is a negative regulator in anthocyanin biosynthesis, but because the results were not the same in all experiments further studies might be needed. The expression levels of *TRY* followed mostly the same pattern as the expression levels of *MYBL2* and TRY is therefore a candidate as an inhibitor in anthocyanin production. The expression levels of *CPC* did not follow the same trend as its homologous *MYBL2* and *TRY* and CPC might therefore not have a role as a negative regulator in anthocyanin synthesis. *CPC* showed the same trend as *DFR* and could therefore be a promoter in this process. Further studies must though be made before a conclusion is made.

The expression of other R3 MYB genes *(TCL1, ETC1, ETC2* and *ETC3* (*CPL3*)) showed interesting trends, that were similar to the expression of *CPC, MYB* and T*RY*. These genes might have a role in anthocyanin biosynthesis but further studies must be performed to achieve a better understanding and before a conclusion of their roles can be made.

6 References

http://nsf.gov/pubs/2002/bio0202/model.htm

http://arabidopsis.org/

Dixon, R. A., Pasinetti, G.M. (2010). "Flavonoids and isoflavonoids: from plant biology to agriculture and neuroscience." Plant Physiol **154**(2): 453-457.

Dubos, C., Le Gourrierec, J., Baudry, A., Huep, G., Lanet, E., Debeaujon, I., Routaboul, J.M., Alboresi, A., Weisshaar, B., Lepiniec, L. (2008). "MYBL2 is a new regulator of flavonoid biosynthesis in Arabidopsis thaliana." Plant J **55**: 940-953.

Feyissa, D. N., Lovdal, T., Olsen, K.M., Slimestad, R., Lillo, C. (2009). "The endogenous GL3, but not EGL3, gene is necessary for anthocyanin accumulation as induced by nitrogen depletion in Arabidopsis rosette stage leaves." Planta **230**(4): 747-754.

Gonzalez A, Zhao M, Leavitt JM, Lloyd AM. (2008). "Regulation of the anthocyanin biosynthesis pathway by the TTG1/bHLH/MYB transcriptional complex in Arabidopsis seedlings." Plant J. **53**(5): 814-827.

Lea, U. S., Slimestad, R., Smedvig P, Lillo C. (2007). "Nitrogen deficiency enhances expression of specific MYB and bHLH transcription factors and accumulation of end products in the flavonoid pathway." Planta **225**(5): 1245-1253.

Lillo, C., Lea, U.S., Ruoff, P. (2008). "Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway." Plant Cell Environm. **31**(5): 587-601.

Lovdal, T., Olsen, K.M., Slimestad, R., Verheul, M., Lillo, C. (2010). "Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato." Phytochemistry **71**(5-6): 605-613.

Matsui, K., Umemura, Y., Ohme-Takagi, M. (2008). "AtMYBL2, a protein with a single MYB domain, acts as a negative regulator of anthocyanin biosynthesis in Arabidopsis." Plant J **55**(6): 954-967.

Misson, J., Raghothama, K.G., Jain, A., Jouhet, J., Block, M., Bligny, R., Ortet, P., Creff, A., Sommerville, S., Rolland, N., Doumas, P., Nacry, P., Herrerra-Estrella, L., Nussaume, L., Thibaud, M-C. (2005). "A genome-wide transcriptional analysis using Arabidopsis thaliana Affymetrix gene chips determined plant responses to phosphate deprivation." Proc Natl Acad Sci U S A **102**(33): 11934-11939.

Morcuende, R., Bari, R., Gibon, Y., Zheng, W., Pant, B.D., Bläsing, O., Usadel, B., Czechowski, T., Udvardi, M.K., Stitt, M., Scheible, W.R. (2007). "Genome-wide reprogramming of metabolism and regulatory networks of Arabidopsis in response to phosphorus." Plant Cell Environ **30**(1): 85-112.

Olsen, K. M., Lea, U.S., Slimestad, R., Verheul, M., Lillo, C. (2008). "Differential expression of four Arabidopsis PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis." J Plant Physiol **165**(14): 1491-1499.

Pelletier, M. K., Murrell J.R., Shirley, B.V. (1997). "Characterization of flavonol synthase and leucoanthocyanidin dioxygenase genes in Arabidopsis. Further evidence for differential regulation of "early" and "late" genes." Plant Physiol **113**(4): 1437-1445.

Ramsay, N. A., Glover, B.J. (2005). "MYB-bHLH-WD40 protein complex and the evolution of cellular diversity." Trends Plant Sci **10**(2): 63-70.

Scheible, W. R., Morcuende, R., Cxzechowski, T., Fritz, C., Osuna, D., Palacios-Rojas, N., Schindelasch, D., Thimm, O., Udvardi, M.K., Stitt, M. (2004). "Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen." Plant Physiol **136**(1): 2483-2499.

Wada, T., Kurata, T., Tominaga, R., Koshino-Kimura, Y., Tachibana, T., Goto, K., Marks, M.D., Shimura, Y., Okada, K. (2002). "Role of a positive regulator of root hair development, CAPRICE, in Arabidopsis root epidermal cell differentiation." Development **129**(23): 5409-5419.

Wang, S., Hubbard, L., Chang, Y., Guo, J., Schiefelbein, J., Chen, J.G. (2008). "Comprehensive analysis of single-repeat R3 MYB proteins in epidermal cell patterning and their transcriptional regulation in Arabidopsis." BMC Plant Biol **8**: 81.

Zhang, F., Gonzalez, A., Zhao, M., Payne, C.T., Lloyd, A. (2003). "A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis." Development **130**(20): 4859-4869.

Zhu, H. F., Fitzsimmons, K., Khandewal, A., Kranz, R.G. (2009). "CPC, a single-repeat R3 MYB, is a negative regulator of anthocyanin biosynthesis in Arabidopsis." Mol Plant **2**(4): 790-802.

Part B: The influence of nitrogen on compounds and quality of tomato

1 Introduction

1.1 Background and aim

Fruits and vegetables are important sources of vitamins, minerals, antioxidants and fiber in the human diet. Tomato (*Solanum lycopersicum*) is one of the most popular and widely consumed fruit (vegetable crop) in the world and is consequently playing a key role in the human diet.

The share of Norwegian greenhouse tomatoes against imported tomatoes is gradually decreasing. In 1995, about 70% of tomatoes sold in Norwegian stores were produced in Norway. In the year 2005 this number had fallen to only 30% (NGF statistic).

The future of Norwegian greenhouse production will depend on how well the producers are able to meet the demands of the consumers. Greenhouse production is more costly in Norway than in other countries like the Netherlands and Spain. The labor cost is high and due to colder climate the heating of greenhouses is a significant cost in the production. It is therefore important for Norway to focus on quality and marketing (Alfnes et al, 2010).

The marked for fruits and vegetables is constantly changing. Most grocery stores were selling only one tomato assortment 15 years ago, but are now selling at least 5 different varieties (Alfnes et al, 2010). Supermarkets are investing more in right presentations of fruits and vegetables and different assortments as cherry tomatoes, truss tomatoes, cocktail tomatoes, plum tomatoes, aroma tomatoes and organic tomatoes. The quality of the fruits is variable so tomatoes are imported to meet the needs of the consumers. But according to MMI reports, Norwegian consumers prefer Norwegian vegetables that are presumed to be of high quality. Norwegian tomatoes were only available in a part of the year but Bioforsk Vest Særheim has developed a production system for year round tomato production using artificial light (Verheul, 2012).

Taste and appearance are qualities that are important for the consumers, along with beneficial effect on human health. It is commonly accepted that the flavor and quality of many fruits, tomatoes included, have declined over the last decades (Klee, 2010). One of the reasons could be that the marked has mostly been focusing on appearance while taste, aroma and health beneficial effects have received less attention. The focus has also been more on yield than quality. Producers are paid by kg product. Their focus is therefore more on how many kg they can produce then on the quality

factors. The flavor of tomatoes is a function of both taste and aroma of several compounds. Important taste parameters are sweetness, acidity, saltiness, bitterness and aroma. Sugars and acids are the main factors contributing to sweetness and sourness of tomatoes (Stevens et al., 1979). It has been shown that Norwegian consumers appreciate varieties with a higher content of soluble solids (sugars), titratable acidity and firmness (Verheul, 2003).

This experiment is a part of a project where the main goal is to increase the consumer's preference for Norwegian greenhouse tomatoes. The aim of this part of the project was to see how nitrogen and light affect quality and compounds in cherry tomatoes. The focus was on how these factors influence firmness of the fruits along with sugars and acids which are the main contributors to taste. Interactive effects of these environmental factors were studied to establish predictable differences in taste and quality. It was also of interest to see if these factors influence the yield of the plants.

Information gained in this research can give the producers access to knowledge they can use to choose the right variety and/or improve the cultivation conditions in their greenhouses to meet the demands of the consumers.

1.2 Tomato fruit development

There are five identifiable stages in tomato fruit development: anthesis, fertilization, cell division, cell expansion and ripening (Fig. 1). This development is regulated by changes in endogenous and external environmental signals. When the fruit is formed cell division occurs which can take place for 7 to 20 days, depending on the cultivar. After that the final fruit cell number is set. The next phase begins after the cell division ceases. The growth of the fruit continues, mostly by cell expansion due to the vacuolar storage of photosynthesis and water. Starch is synthesized and stored in the fruit. This phase continues until the fruit has reached its final size. The last stage is ripening, which is controlled by the enzyme ethylene and ripening related transcription factors. During ripening, the tomatoes undergo several metabolic transformations like importation and accumulation of sugars, starch degradation and synthesis of lycopene and carotenoids. Chlorophylls are degraded and the cell wall softens (Gillaspy et al., 1993; Beckles et al., 2011 and Fujisawa et al. 2011).

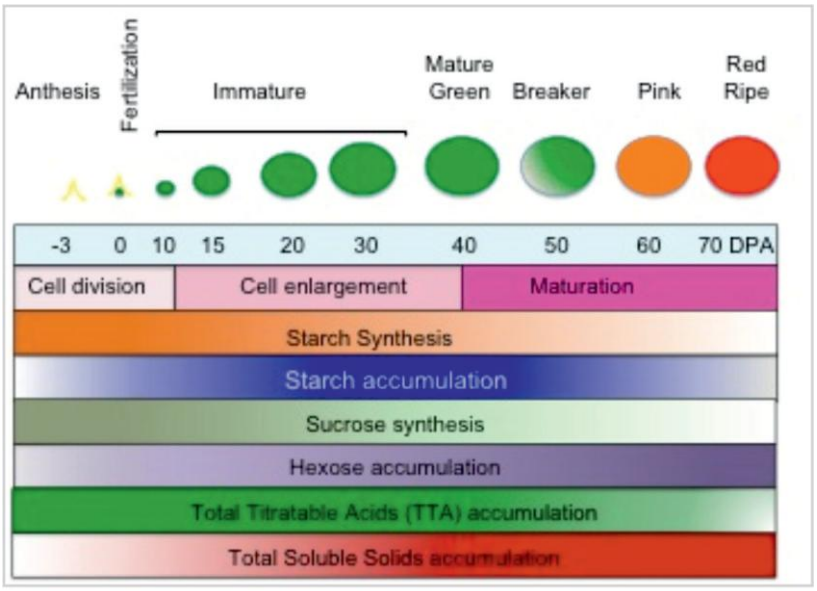


Figure 1. Changes in physiology and carbohydrate accumulation during tomato fruit development. The classification as "Immature to Red Ripe" are based on USDA Standards. Days past anthesis (DPA) are based on S. Lycopersicum L and other cultivars. The picture is taken from Beckles et al. 2011.

1.3 Firmness

Firmness and texture of tomatoes are important elements for the consumers to evaluate the fruit quality. There are several factors that control the fruit firmness like the elasticity of the pericarp tissue. The ripening of the fruit, including the softening of the fruit cell wall, is regulated by numerous genes and the mechanism is complex and unclear (Wann et al. 1996 and Fujisawa et al. 2011). The consumers demand for a better quality promotes the producers to grow tomato plants that bear fruits that stay firm during post-harvest.

1.4 Compounds in tomatoes

Tomatoes are an important source of minerals, vitamins and antioxidant compounds and their quality can be divided into flavor (organoleptic) and health beneficial (nutraceutic) values. Minerals, vitamins, carotenoids and flavonoid compounds contribute to the nutraceutical quality. Carotenoids include vitamin A and antioxidant agents, and can therefore play a role in preventing diseases like heart diseases and cancer. Flanvonoids along with compounds like ferulic, chlorogenic and cafeic acid, seem to have the same beneficial effects on human health. Tomatoes have also a small quantity of vitamin E and glycoalkaloids. Sugars, acids and volatile compounds contribute to the organoleptic quality (Dorais et al. 2001).

The soluble dry matter of tomatoes (Soluble Solid Content, SSC) consists mainly of sugars and acids, sugars being the dominant content. The sugars are mostly the reducing sugars fructose, glucose and also a small amount of sucrose. Studies have shown that the levels of sugars and acids in tomatoes affect the taste attributes of sweetness and sourness respectively, and are also major factors in overall flavor intensity (Malundo et al. 1994 and Stevens et al. 1979).

Sugar is the major photoassimilate and is transported from photosynthetic leaves to the growing fruit via osmotic pressure gradient. Sucrose can be transported to the fruit cells via the symplast (through the plasmodesmata) or the apoplast and there are 8 enzymes that are involved in the process (Fig. 2). Invertase are enzymes in the apoplast that convert sucrose into hexoses which are then imported into the cell by apoplastic hexose transporters that are located on the plasma membrane. In the cytoplasm, sucrose is converted to fructose and UDPglucose by sucrose synthase (Susy) or into gucose and fructose by neutral cytoplasmic invertase (Fig. 2). Sucrose formed by invertases is most likely transferred to the vacuole to be stored there. The activity of Susy, along with the hexokinases, probably mobilizes carbon from the sucrose to the hexose-P-pool to be used in starch production (Beckles et al. 2011 and Steinhauser et al. 2010).

Starch is synthesized from hexose phosphates while the fruit is still green, or from anthesis until 13 days past anthesis (DPA). Accumulation of starch is maximal around 40 DPA and is then degraded during ripening. By fixing these sugars as starch, sucrose import into the cell might be enhanced (Luengwilai et al. 2009). During ripening, starch biosynthesis becomes minimal and degradation of starch begins. Invertase and apoplastic import of hexose increases with storage of sugars in the vacuola (Beckles et al. 2011).

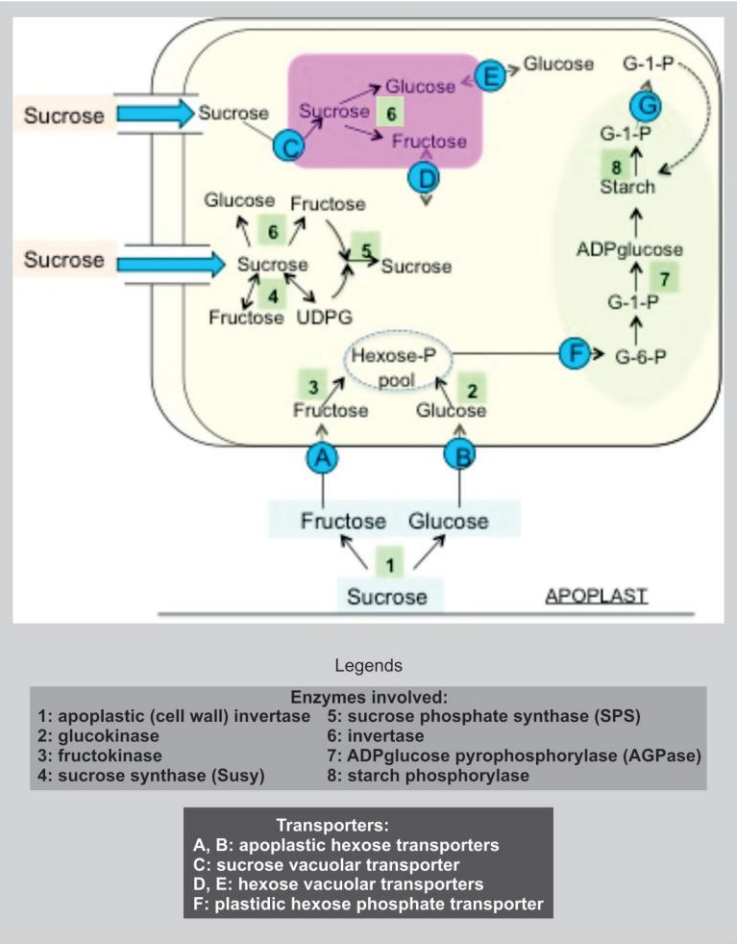


Figure 2. Import of sucrose and carbohydrate metabolism in tomato fruit. Sucrose is imported via apoplast or symplast. The synthesize of starch starts early in the fruit development and he accumulation is maximal around 40 days past anthesis (DPA). The picture is taken from Beckles et al. 2011

The major acid in tomatoes is citric acid but there is also a slide quantity of malic acid and glutamic acid (Malundo et al. 1994 and Anthon et al. 2011). It has been seen that the acid concentration increases when the fruit color changes from green to pink and further to red stage. The acid concentration is at its maximum point when the fruit starts to ripen and decreases during ripening. In contrast to the acids, sugar contents increase in tomatoes during ripening (Gautier et al. 2008 and Anthon et al. 2011). The decrease in acid concentration is mostly due to loss of citric acid (Anthon et al. 2011).

1.5 Fertilizers

Nutrition is a complex process involving many nutrients that are important for the plant to grow. Different cultivars of crops, like tomatoes, may respond differently to a particular nutritional status. External factors like environmental conditions can also affect how crops respond to nutrients.

Compounds that are usually given to horticultural plants are Nitrogen (N), Potassium (K), Phosphorus (P), Calcium (Ca) and Magnesium (Mg) (Peet et al. 2005).

Nitrogen availability in the nutrient can affect visual quality and taste of fruits and vegetables. Sufficient amount is necessary for the plants to grow and develop normally but to high nitrogen level can lead to extended vegetable growth that can cause deletion in fruit development. Very low nitrogen level can cause weaker and paler plants with declined fruit yield and quality (Peet et al. 2005). Nitrogen can be supplied to plants in both anionic (NO_3^-) and cationic (NH_4^+) form. Earlier studies have shown that the form of nitrogen provided to plants may influence their growing situation and their availability to take up other nutrients. It can also affect the plant metabolism. High fraction of ammonium to the total nitrogen supply can for example change the pH of the rhizosphere and influence the plants uptake of P and micronutrients. It can also change the calcium balance within the fruit causing more incidence of plants with diseases like blossom-end rot (BER) (Passam et al. 2007). In the present study, the main nitrogen source was in NO_3^- form.

Potassium (K) is another important compound given to horticultural plants. It regulates growth of the plants and is required to get a better quality of the fruits. The ratio between potassium and nitrogen (K/N) is significant for the growth of the plant, where high ratio leads to slower growth (Peet, M.M. 2005).

1.6 How to improve quality and taste of tomatoes

The quality of tomatoes can be determined by appearance of the fruit like color, size and firmness and can be affected by growth conditions along with different varieties (Dorais et al, 2001). Growth situations in greenhouses can influence quality related compounds like taste, aroma, flavonoids and carotenoids of the fruit and the impact can be so significant that differences in tomatoes grown in Norwegian greenhouses have been shown to vary more between the producers than in variety (Verheul, 2004). Climate factors (temperature, CO_2, humidity, light), nutrition (composition, electrical conductivity) and management practices (plant density, choice of variety, leaf/fruit ratio, harvest date) can influence directly and indirectly the accumulation and degradation of quality related compounds in tomatoes. These factors can be regulated in greenhouse production and a better control and understanding on managing these factors can have a good impact on improving taste and quality of tomatoes and other fruits or vegetables.

Many studies have been made to get a better understanding on how to produce tomatoes with better taste and more quality. Earlier researches on the effects of environmental factors have given contrast results, probably due to complex interaction between these factors. It has been shown that light and plant nutrition are the main factors that affect the accumulation of quality related compounds (Slimestad et al. 2005).

Electrical conductivity (EC) can affect the quality of tomatoes. Under high EC, water becomes less available for the plants (osmotic effect) reducing the transport of water and solutes by the xylem. It has been shown that by inducing salinity in the soil, resulting in higher EC, the organoleptic quality of the fruit is improved. The disadvantage is that it often reduces fruit size and diminishes marketable yield (Dorais et al, 2001a).

Temperature can influence the movement of photoassimilates in plants which can affect the growth of the plants along with the fruits they bear. It has an effect on the fruit metabolism and therefore on compounds that determine the quality of the fruit, like color, texture, size and organoleptic properties. High temperature increases the vegetative growth of the plant and its fruits reducing their quality as a consequence. Too low temperature slows down fruit ripening and favors irregularly shaped fruits (Dorais et al. 2001b). Gautier et al. (2008) examined how tomato quality varies with increased temperature. They noticed that secondary metabolites were affected but sugars and acids did not respond significantly.

Like all plants, tomatoes need light to grow and to produce fruits. According to earlier researches, tomatoes need a minimum light of 4 mol/m^2/day for the production and development of the fruits. (Heuvelink et al. 2003). There has also been found a linear relationship between tomato growth and cumulative light where the growth induces in more light (Cockshull, 1988). Supplemental light can therefore be used to increase tomato crop growth but it is uncertain if it affects the quality of the tomatoes. Gautier et al. (2008) found for example that irradiance did not affect the final composition of sugars and acids at the ripening stage. Other compounds with antioxidant properties where on the other hand modified considerably.

Different levels of nitrogen in the nutrition can be used to improve the quality of tomatoes. The ratio between potassium and nitrogen is important and can be changed to influence the final combination of compounds during ripening stage. Very high amounts of nitrogen in the nutrition can lead to fruits with poor taste due to lower sugar content and increased fruit acid concentration (Locascio et al. 1994). Less is known about how low nitrogen concentration affects the sugar/acid ratio in tomatoes. Dorais et al (2001b) showed that a very low concentration of nitrogen reduced the size, color and the yield. This was because the concentration of nitrogen influenced the development of the foliar

canopy and therefore the quantity of photo assimilates available to the fruits. It must be noticed though that this experiment was done under natural light so it would be desirable to see how additional light affects these results.

Nitrogen levels can also be used as tools to influence plants to produce desirable contents like anthocyanin and other phenolics, which are compounds thought to have a health beneficial effects on humans. Phenolics are also involved in the plants resistant to UV light and pathogens. A high level of phenolics could therefore lower the need for pesticides (Lillo et al. 2008; Clé et al. 2008) Løvdal et al. (2009) showed how stress factors like lack of nitrogen, along with low temperature enhanced anthocyanin production in tomatoes. The accumulation of this compound was mainly in the leaves of the plants, but not much in the fruits. Larbat et al. (2011) observed an increase in leaf phenolic concentration after short N-limitation periods. The N limitation had a negative impact on the relative growth rate.

It is known that higher sugar content in the fruit influences consumer fruit likeability. But the fruits are bred for optimal yield and postharvest handling which often leads to loss of soluble solids like sugars. The fruits are usually harvested before full-ripe stage is reached which cuts of the sugar supply to the fruit. One solution to this problem is to grow tomatoes that produce more sugars to minimize the effect on sugars and without yield loss (Beckles et al. 2011). Focus has been, for example, on the biochemical pathways like starch biosynthesis. During ripening, starch is degraded and sugar (glucose and fructose) is imported to the fruit. Wild tomato species have been found to contain higher level of sugars during ripening and genetic, molecular and biochemical characterization of the species have given a better insight into carbohydrate metabolism in tomato which can be used in breeding studies (Knapp et al. 2004). Some wild species store more sugars then the cultivated tomato (*S. lycopersicum*) and many high – TSS (total soluble solids) wild tomato species accumulate more starch (Yelle et al. 1991 and Fridman et al. 2004). Wild tomatoes with high sugar content are usually low-yielding. It is desirable for the tomato industry to break this barrier and produce fruits that contain the high-yielding property from cultivars and the high sugar content from wild types. The tomato line Solara was developed by crossing the cultivar (*Solanum lycopersicum* L) and the wild type *S. pimpinellifolium*. Horticultural yield was 30 % higher in Solara compared with *S. lycopersicum* L. The fruits had also higher TSS than most cultivars (Beckles et al. 2011). However, engineering horticultural varieties for higher yield and better quality can be difficult and a better knowledge of metabolic fluxes and enzyme activity are important to understand the roles of various enzymes in fruit metabolism to be used to improve the quality of cultivated tomatoes.

As can be seen, a large amount of literature reports on environmental effects on accumulation and degradation of compounds in tomatoes related to quality. Less attention has been on how to control these environmental effects. Earlier results are often in contrast with each other presumable due to complex interaction. Verheul et al. (2012) has developed a taste model which can be used to measure the quality of tomatoes on a consumer scale (unpublished data). By using this model along with a better understanding of environmental factors affecting horticultural tomatoes, the producers can evaluate how they can improve their greenhouse production to be better able to meet the needs of the consumers.

2 Material and methods

2.1 Plant material

Fruits were sampled from four tomato (*Solanum lycopersicum*) cultivars, cv Susanne, Juanita, Tastery and Claree, all cherry type tomatoes with different genetic background. The plants were planted on 13.6.11 and grown on rock wool at a plant density of 3.5 plants m^{-2} in two greenhouse compartments. The CO_2 level in the air was 800 ppm and the vapor pressure deficit was minimal 2.8%. The temperature was adapted to the cultures (Fig. 5). The tomatoes in one of the greenhouse compartments received natural light while the other compartment was supplemented with light from high pressure sodium (Philips HPS – SONT – 400 W) lamps (240 µmol $m^{-2}s^{-1}$ photosynthetic active radiation, 18h day-1 when global radiation outside < 275 W m^{-2}). The plants were watered sufficiently with standard nutrition for 6 weeks before the treatment started. Two repetitions were made for each treatment.

Before the treatment began the plants received the following nutrition with 30 % runoff:

Table 1. Standard nutrition for tomato plants. gram/1000 liter solution.

	gram	N	P	K	Ca	Mg	S	Si	Cl
pioner red (azelis.com)	1046	91	47	314		37	47		
Calcium nitrate	1026	159			195				
Magnesium Sulfate	1000					16			
Water		0	0	0	0	0	0	0	0
Promille		**250**	**47**	**314**	**195**	**53**	**47**	**0**	**0**
Composition ratio		100	19	126	78	21	19	0	0

Electrical conductivity (EC) can be seen in Table 2.

Table 2. Electrical conductivity. gram/1000 liter solution

	gram	EC
pioner red (azelis.com)	1046	1.05
Calcium Nitrate	1026	1.03
Magnesium Sulfate	1000	1.00
Water		0.00
Promille		**3.07**
Composition ratio		

The experimental design was a randomized block design with two light intensities, four tomato varieties, four nutrient compositions, and two parallels, each consisting of five plants. Treatments with different nutritient compositions startet 15.8.11, just after the start of harvesting of the third truss. In the nutrient solutions, the ratio between K and N was enhanced by using different nitrogen levels.

1) Control solution, K/N = 0.5
2) K/N = 7
3) K/N = 3
4) K/N = 2

After the start of the treatments, the tomatoes were harvested for 10 weeks. Samples were collected according to standard production procedure with orange-yellow colored fruits (color scale 6). Color was determined visually using a scale from 1 (green) to 12 (deep red) (Colorscale Vekstmiljø/NORGRO). Six tomatoes were harvested from each treatment (12 tomatoes for Susanne (control plant)). Tomatoes were picked from the middle of the cluster where orange-yellow tomatoes are usually located. The first and last 4 tomatoes on the truss were avoided because of different sugar/acid content in those fruits. The weight was registered and the samples were put in a dark chamber at 12°C. The firmness of the fruits, Soluble Solid Content (SSC in °Brix) and titratable acidity (TTA) was measured within two days after harvesting.

For yield registration, tomatoes were harvested twice a week and the no. of fruits and average fruit weight were registered. Yield was calculated by multiplying number of fruits harvested with average fruit weight.

Data analysis was performed using the General line model (Anova) in the software Minitab 16.

2.2 Physical analysis

The firmness of the fruit was measured with Durofel DFT 100. Three tomatoes were randomly picked from each sample and the firmness was measured at two opposite points on each fruit. The firmness was given in scale 1 – 100 where each unit was 0.025 mm.

2.3 Chemical analysis

A digital Refractometer PR-101 α (Atago, Japan) was used to measure Soluble Solid Content (SSC) of the tomatoes. Each sample was crushed in a food processor and a drop from the sample was put on the sensor of the refractometer to measure SSC content. SSC was expressed in °Brix where for example unit 10 equaled 10 % soluble solids in the solution.

Titratable acidity (TTA) was measured using 794 Basic Titrino (Metrohm Switzerland), where 0.1 M NaOH was used to determine the amount of acids in the tomato samples. Crushed tomatoes (5 mg) were put in a glass beaker and distilled water added (about 1/5 of the beakers volume). The beaker was then put on a stirrer and a pH sensor and a dripper dipped into the solution. The equilibrium point (EP) at pH 8.0 – 8.5 was used to calculate TTA of the tomatoes. Before measuring the samples, 794 Basic Titrino was calibrated by using ca 67 mg citric acid. TTA was expressed in equivalents (mg CAE 100^{-1} g FW).

3 Results

Plants receiving treatment 4 (K/N = 2) were grown nearest the edges of the greenhouse compartments where the growing situation was more affected by the environment outside. This was considered to influence the plants and possibly bias the results. It was therefore decided not to include these plants in statistics and other calculations. The data can be seen in Table B1 in Appendix B.

3.1 Environmental conditions, light and temperature

The experiment was done in late summer to winter 2011 (august – oktober). The radiation was quite low at this time, especially in September to November where the global radiation was almost entirely under 10 MJ m-2s-1 (Fig. 4). Average daily temperatures in the two greenhouse compartments are shown in figure 5. Temperatures were adapted to the cultures with and without use of additional light. It is shown that a higher temperature was achieved in the compartment with additional light. Average temperature decreased during the season, especially in the compartment without artificial light. The temperature was quite stable in the greenhouse compartment with additional light but fluctuated a bit in the growing chamber with natural light (Fig. 5).

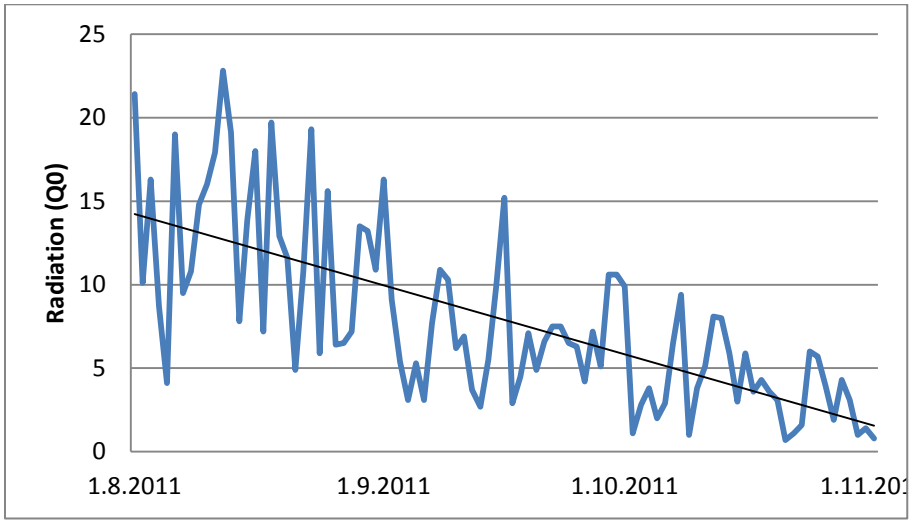


Figure 4. Outside global radiation (MJ m-2s-1) measured at Bioforsk Vest Særheim 1. august – 1. november 2011.

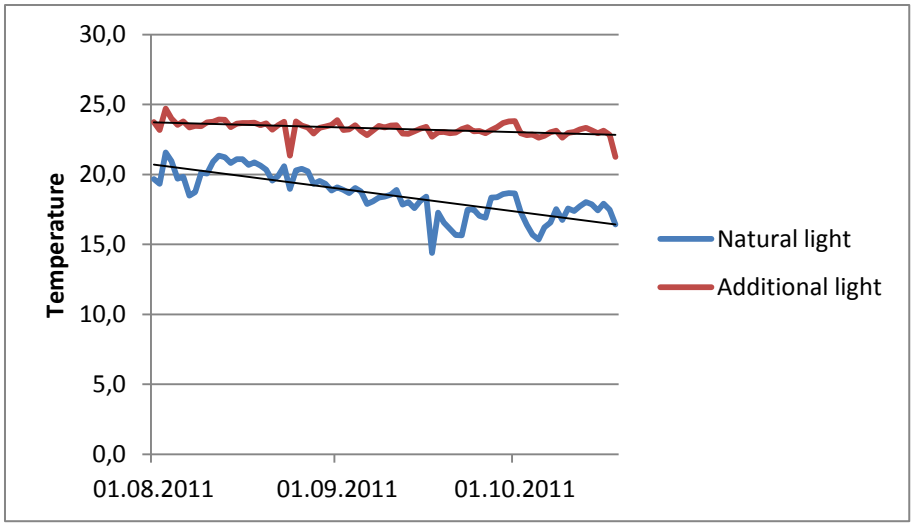


Figure 5. Average daily temperature in two greenhouse compartments at Bioforsk Vest Særheim .

3.2 Factors affecting the quality and taste of tomatoes

Main effects and interactions of harvesting week number, additional light (with or without), type (cultivar Claree, Susanne, Juanita and Tastery) and nutritional composition (Treatment K/N = 0.5, K/N = 3 and K/N = 7) on weight, firmness, soluble solid content (SSC), titratable acidity (TTA) and taste were measured and grouped using Tukey method. Values followed by different letters are significantly different. The effects are statistical significant if the critical p – value is lower than 0.05. The marketable value of the tomatoes was calculated as fruit soluble solids per m^2 and the same measurements were performed as for the other parameters (Table 3).

Table 3. Grouping information using Tukey method and 95% confidence for weight, firmness, SSC, TTA, taste.and SSC*Yield. Analysis for interaction effects for weight, firmness, SSC, TTA and taste. *; p<0,05, **; p<0,01, ***; p<0,001

	Fruit weight (g)	Firmness	SSC (Brix)	TTA (mg CAE 100^{-1} g FW)	Taste (0 – 100)	SSY/g p m^2
Week						
33	14.4 (a)	68.9 (d)	5.8 (a)	699.7(bc)	38.4 (ab)	75 (a)
34	12.4 (b)	70.7 (cd)	5.4 (b)	729.9(abc)	33.7 (b)	65 (b)
35	12.0 (bc)	69.6 (d)	5.9 (a)	703.5(abc)	40.6 (a)	52 (c)
36	10.9 (cd)	71.7 (bc)	5.9 (a)	761.3 (a)	41.7 (a)	49(cd)
37	10.1 (de)	72.1 (bc)	5.8 (a)	709.4(abc)	40.1 (a)	42(de)
38	9.5 (e)	72.5 (ab)	5.8 (a)	757.5 (ab)	40.2 (a)	36 (ef)
39	9.3 (e)	72.6 (ab)	5.8 (a)	709.1(abc)	40.3 (a)	34 (fg)
40	9.1 (e)	72.8 (ab)	5.8 (a)	669.3 (c)	38.2 (ab)	33 (fg)
41	9.2 (e)	73.9 (a)	5.6 (ab)	745.1 (ab)	36.6 (ab)	27 (g)
42	9.0 (e)	73.1 (ab)	5.7 (ab)	696.7 (bc)	38.5 (ab)	3 (h)
Light						
+	10.7 (a)	72.1 (a)	5.9 (a)	675.6 (b)	40.7 (a)	0.5 (a)
-	10.6 (a)	71.5 (b)	5,6 (b)	760.8 (a)	36.8 (b)	0.3 (b)
Type						
Claree	11.1 (b)	64.8 (d)	5.9 (b)	770.6 (a)	41.0 (b)	0.5 (a)
Susanna	9.7 (c)	68.6 (c)	6.3 (a)	798.8 (a)	50.8 (a)	0.5 (a)
Juanita	6.8 (d)	72.6 (b)	5.2 (c)	681.7 (b)	27.5 (d)	0.5 (a)
Tastery	14.7 (a)	81.0 (a)	5.7 (b)	621.6 (c)	35.7 (c)	0.3 (b)
Treatment						
Control (K/N = 0.5)	10.6 (a)	71.3 (b)	5.8 (a)	720.9 (a)	38.9 (a)	0.4 (a)
K/N = 7	10.5 (a)	72.3 (a)	5.8 (a)	744.8 (a)	39.7 (a)	0.4 (a)
K/N = 3	10.7 (a)	71.7 (ab)	5.7 (a)	688.8 (b)	37.7 (a)	0.4 (a)
Week*light	***	n.s.	n.s.	***	n.s.	***
Week*type	**	*	n.s.	n.s.	n.s.	**
Week*treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Light*type	*	**	***	*	**	***
Light*treatment	**	n.s.	*	***	n.s.	***
Type*treatment	n.s.	n.s.	*	n.s.	*	**
Week*light*type	n.s.	n.s.	n.s.	n.s.	n.s.	**
Week*light*treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Week*type*treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Light*type*treatment	n.s.	***	**	n.s.	**	n.s.
Week*light*type*treatment	-	n.s.	n.s.	n.s.	-	-

3.2.1 Fruit weight

As can be seen in figure 6 and table 3, there was not much difference between the average weights of tomatoes grown under natural light and those grown with additional light. However, fruit weight was reduced from the start to the end of the culture (Fig. 6 and 7). Fruits grown with additional light

had tendency to higher fruit weight than fruits grown with natural light, when outside global radiation was low (Fig. 6). Tomato fruits of the cultivar Tastery had highest weight, while the fruits of 'Juanita' were the lightest ones during the culture (Fig. 7 and 8).

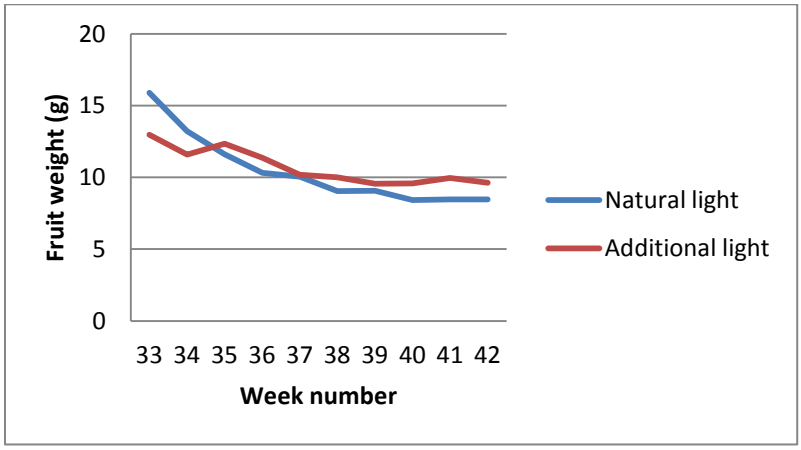


Figure 6. Development of average fruit weight of cherry tomato cultivars grown with or without supplemental light.

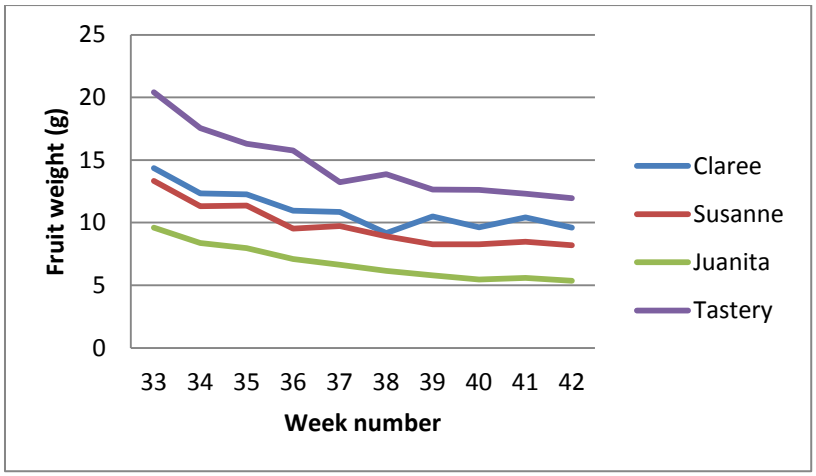


Figure 7. Fruit weight of four cherry tomato cultivars through the experiment (average for two light conditions and four treatments).

.

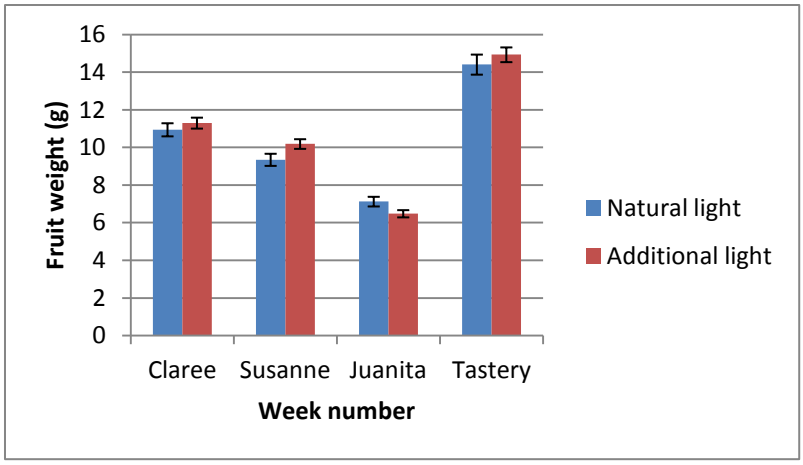


Figure 8. Average fruit weight of four cherry tomato cultivars with or without the use of supplemental light (average for 10 harvesting weeks).

The composition of the nutrient solution had different effects under the two different light conditions. The average fruit weight increased when the plants were grown in additional light and given treatment with the K/N ratio 0.5 compared to plants grown in natural light. In contrast, average fruit weight decreased in additional light when the plants received lower amount of nitrogen (K/N = 7 and K/N = 3) (Fig. 9).

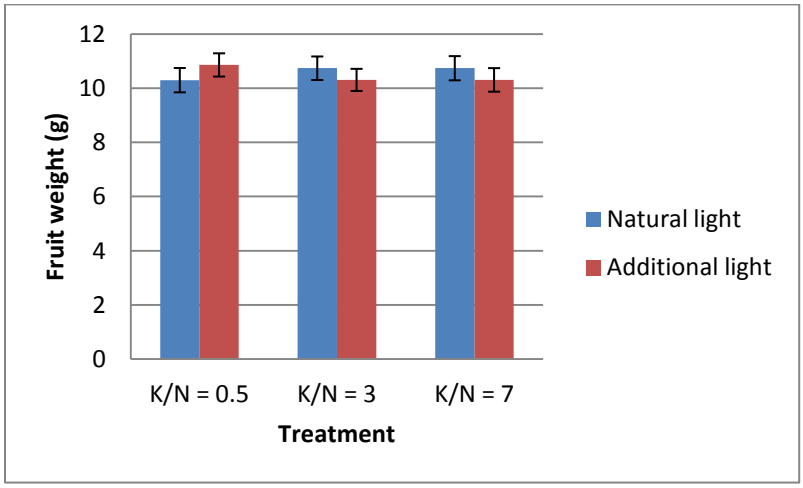


Figure 9. Fruit weight of cherry tomato grown with three different nutrient solutions with or without the use of artificial light (average of four cultivars and 10 harvesting weeks).

3.2.2 Firmness

Fruit firmness was different for the different tomato types during the experiment. The firmness of each type did not change considerable during the weeks but the difference can be seen between the sorts, 'Tastery' being the firmest one and 'Claree' bearing the softest fruits (Fig. 10).

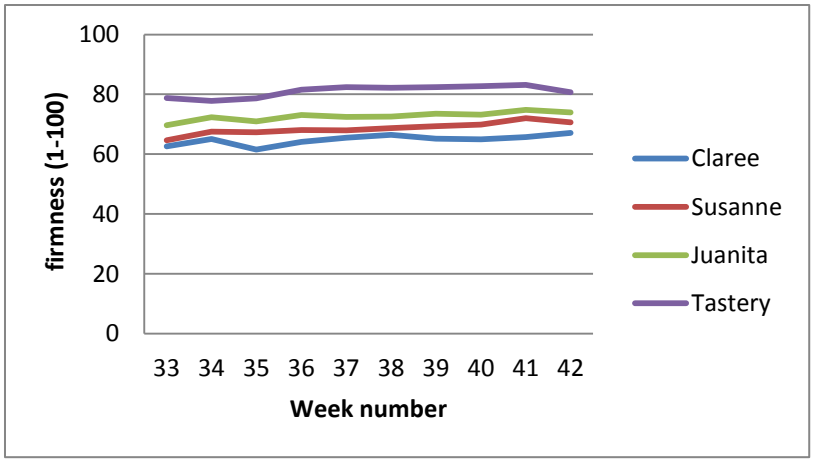
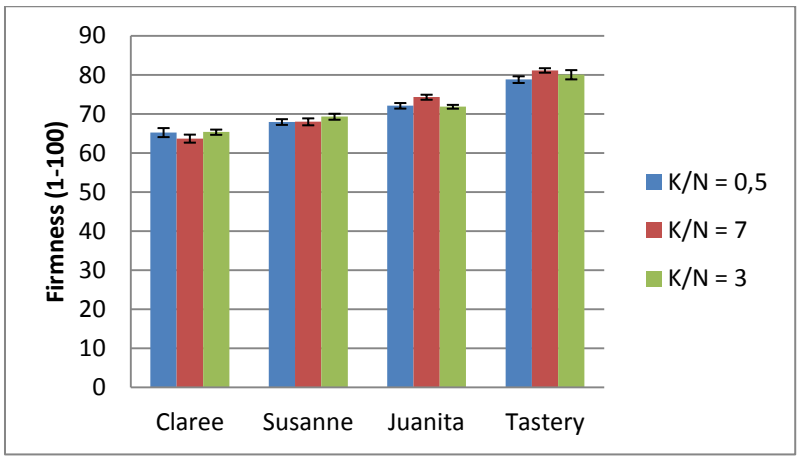


Figure 10. Firmness of four tomato cultivars through the experiment (average for two light conditions and four treatments).

Figure 11 shows the difference in firmness between the tomato cultivars and how different treatments affected the firmness of the tomatoes when grown in different light conditions. The type effects are clear with the fruits from 'Tastery' being the firmest ones. There is no clear difference between the treatments, but Susanne responds quite well to K/N = 7 when grown with additional light while 'Juanita' and 'Tastery' respond positively to K/N = 7 in natural light.

(A)



(B)

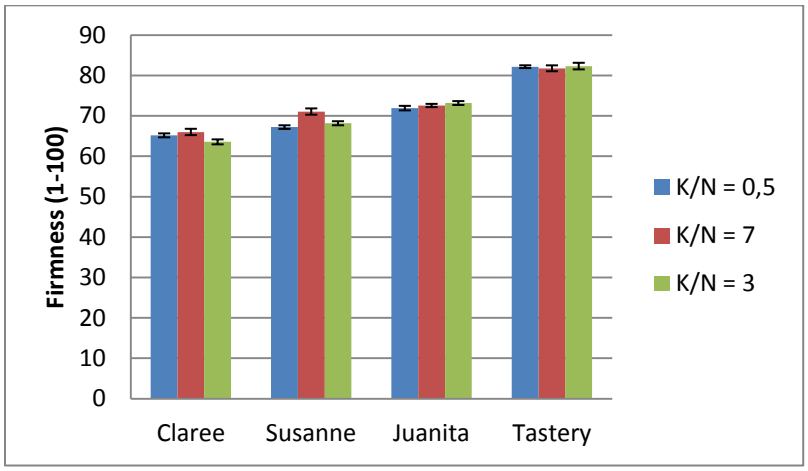
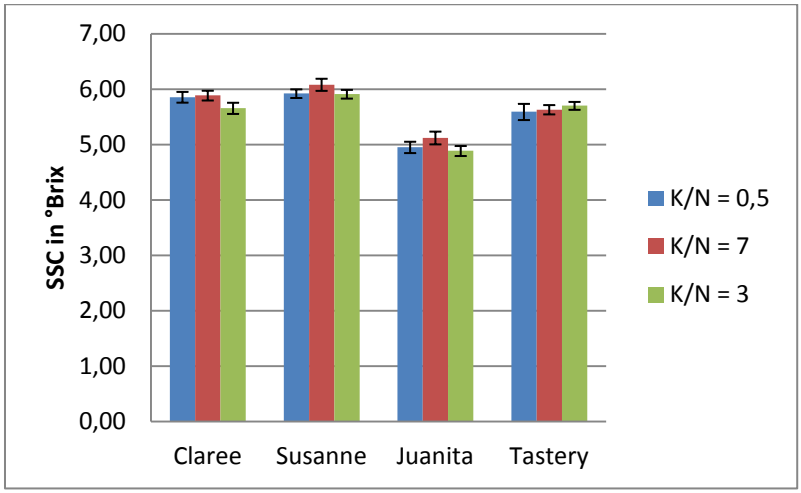


Figure 11. Average firmness for four cherry tomato varieties treated with three different nutrition solutions. (A) Plants grown under natural light conditions. (B) Plants grown supplemented with additional light (240 µmol $m^{-2}s^{-1}$ photosynthetic active radiation, 18h/day when global radiation outside < 275 W/m^2).

3.2.3 Soluble Solid Concentration (SSC)

Little differences were found in the SSC of fruits grown with or without additional light, except for 'Susanne' where a small increase was observed in additional light. 'Juanita' had the lowest concentration of soluble solids. The effects of different treatments were clearer in plants growing in additional light where the plants responded more positively to a higher amount of nitrogen. 'Susanne' and 'Claree' show tendency to higher SSC at K/N = 0.5 when grown with additional light. 'Juanita' had higher SSC content at K/N = 3 when supplemented with extra light. 'Claree', 'Susanne' and 'Juanita' grown in natural light had tendency to higher SSC when low amount of nitrogen was used (Fig. 12).

(A)



(B)

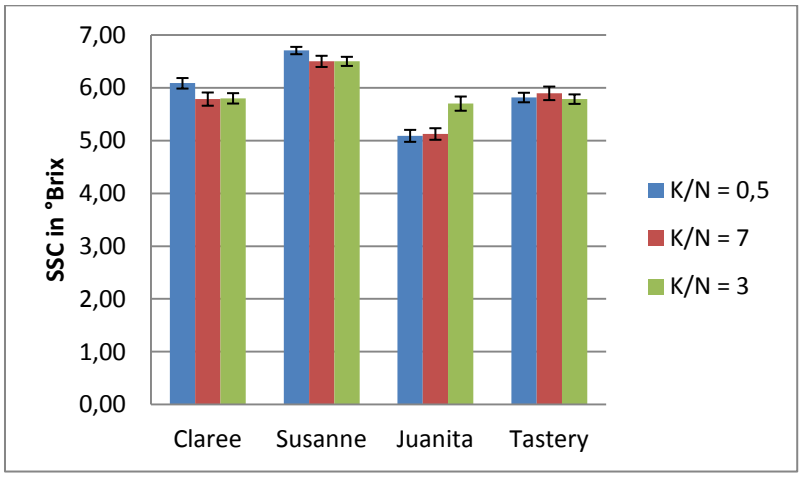


Figure 12. Average sugar concentration in four cherry tomato varieties grown with three different nutrition solutions. (A) Plants grown under natural light conditions. (B): Plants grown supplemented with additional light (240 µmol $m^{-2}s^{-1}$ photosynthetic active radiation, 18h/day when global radiation outside < 275 W/m^2).

3.2.4 Titratable acidity (TTA)

The TTA fluctuated a bit during the experiment period but the final concentration did not differ much from the initial concentration. The plants growing in natural light produced more acids than the tomatoes that received extra light (Fig. 13 and 14). The cultivars Claree and Susanne had higher acid content than 'Juanita' and 'Tastery' (Fig. 14).

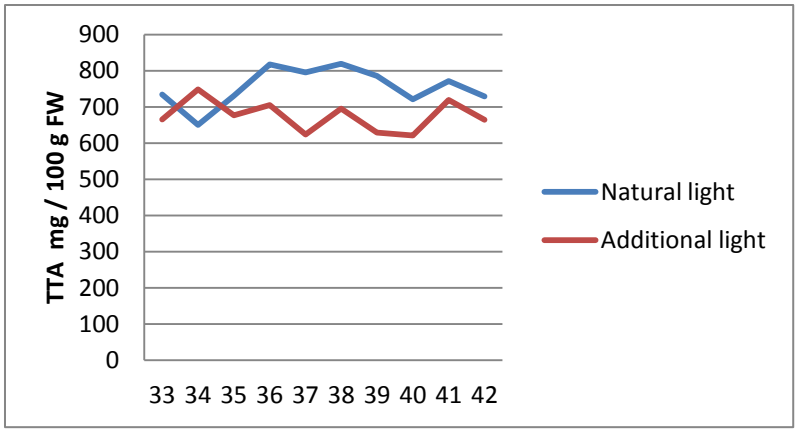


Figure 13. Average acid contents in tomato varieties grown with or without supplemental light.

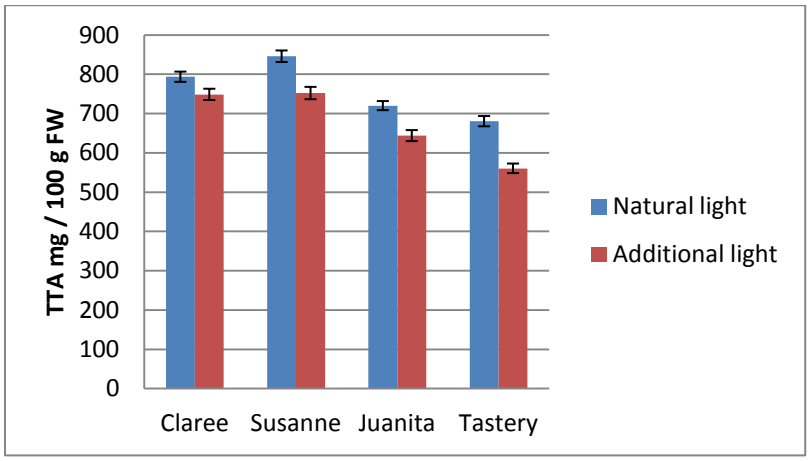


Figure 14. Titratable acidity in four cherry tomato varieties grown in two different light conditions.

There was not much difference between treatments for plants grown under natural light conditions but for plants grown under additional light conditions and treatment 3 (K/N = 3) resulted in plants with less acid concentration (Fig. 15).

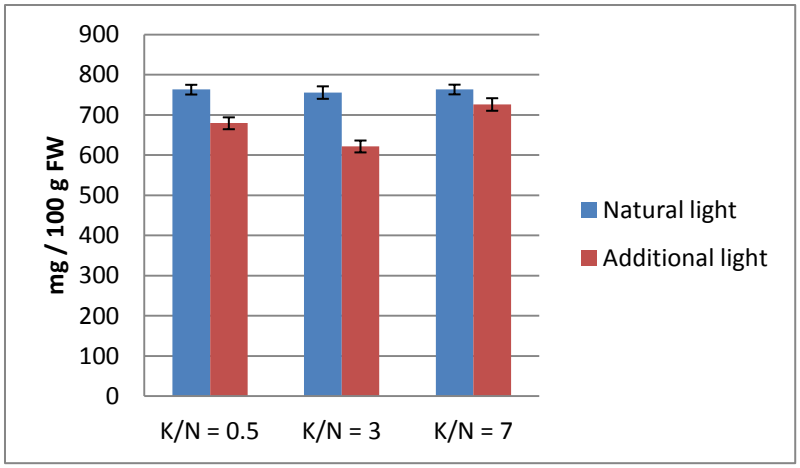
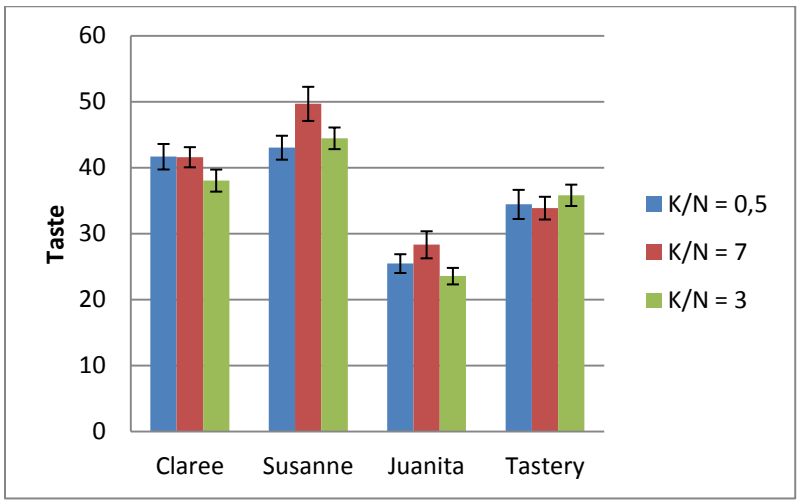


Figure 15. Acid content in tomato varieties treated with three different nutrition solutions and grown in natural light and additional light.

3.2.5 Taste

Results show that treatment effects on taste are dependent on light conditions and cultivars. Under natural light conditions, a better taste is found in 'Susanne' and 'Juanita' at K/N = 7 (Fig. 16). This is confirmed for 'Susanne' in earlier studies. When using additional light, a better taste is found in 'Susanne' and 'Claree' at K/N = 0.5 and in 'Juanita' at K/N = 3.

(A)



(B)

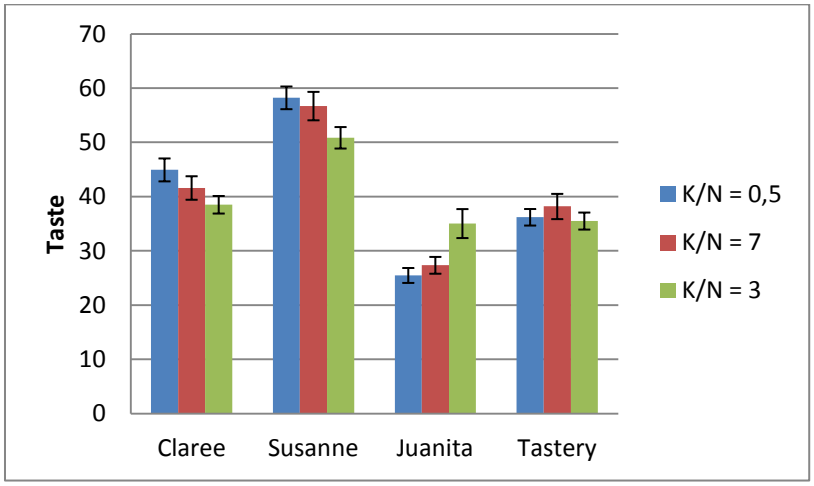


Figure 16. The influence of different nutrient solutions on the taste in four cherry tomato varieties. (A) Plants grown under natural light conditions. (B) Plants grown supplemented with additional light (240 µmol $m^{-2}s^{-1}$ photosynthetic active radiation, 18h/day when global radiation outside < 275 W/m^2).

3.3 Total yield

Main effects and interaction effects of light conditions (with or without supplemental light), tomat type (cv Claree, Susanne, Juanita and Tastery) and Treatment (K/N = 0.5, K/N = 3 and K/N = 7) on the total number of harvested fruits pr m-2 greenhouse surface, the average fruit weight, total yield and soluble solid yield pr m-2 (SSY, g m^{-2}) grouped using Tukey method. The effects were statistical significant if the critical p – value was lower than 0.05 (Table 4).

Table 4. Grouping information using Tukey method and 95% confidence for No. of fruits m^{-2}, fruit weight (g), yield kg m^{-2}, and SSY g m^{-2}. Analysis for interaction effects for No. of fruits m^{-2}, fruit weight (g), yield kg m^{-2}, and SSY g m^{-2}. *; p<0.05, **; p<0.01, ***; p<0.001

	No. of fruits m^{-2}	Fruit weight (g)	Yield kg m^{-2}	SSY g m^{-2}
Light				
+	1200 (a)	12.3 (a)	13.82 (a)	535 (a)
-	661 (b)	12.2 (a)	7.97 (b)	294 (b)
Type				
Claree	981 (a)	12.7 (b)	12.16 (a)	462 (a)
Susanna	974 (a)	11.8 b)	11.31 (a)	459 (a)
Juanita	1060 (a)	8.3 (c)	8.61 (b)	446 (a)
Tastery	704 (b)	16.4 (a)	11.50 (a)	291 (b)
Treatment				
Control (K/N = 0.5)	930 (a)	12.1 (a)	10.89 (a)	427 (a)
K/N = 7	929 (a)	12.1 (a)	10.70 (a)	413 (a)
K/N = 3	932 (a)	12.4 (a)	11.11 (a)	402 (a)
Light*type	***	n.s.	*	*
Light*treatment	n.s.	n.s.	n.s.	n.s.
Type*treatment	n.s.	n.s.	n.s.	n.s.
Type*treatment*light	n.s.	n.s.	n.s.	n.s.

3.3.1. Number of fruits harvested per m^2

As can be seen in figure 17 and table 4, plants grown with additional light produced more fruits than plants grown in natural light. Tastery had the fewest fruits harvested but the difference between the other sorts was less clear. Different treatments did not have significant effect (Table 4).

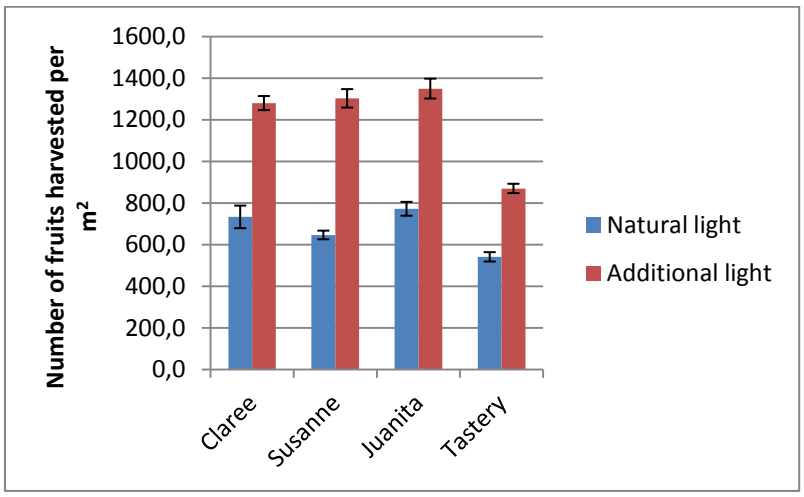


Figure 17. Effects of the use of supplemental light on the total number of harvested fruits per m-2 for four tomato types during 10 harvesting weeks (average of three treatments).

3.3.2 Average fruit weight

Average fruit weight was different for different tomato types (Table 4). There was however no effect of light conditions or treatments with different nutrient composition on the average fruit weight.

3.3.3 Yield

A higher yield was achieved when the plants were grown in additional light (Fig. 18). The same pattern could be seen in soluble solid yield per m^2 (SSY g m^{-2}) (Fig. 19). The yield was lower in 'Juanita' and 'Tastery' than in the other two cultivars when supplemented with extra light (Fig. 18 and 19).

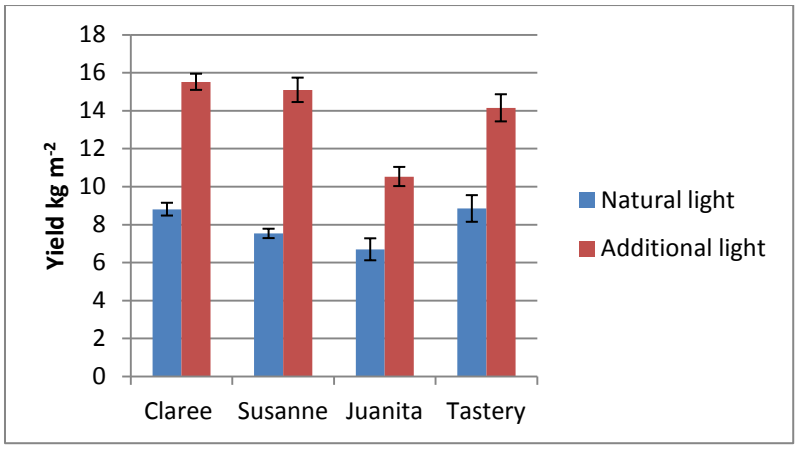


Figure 18. The influence of additional light on the total yield of four cherry tomato varieties.

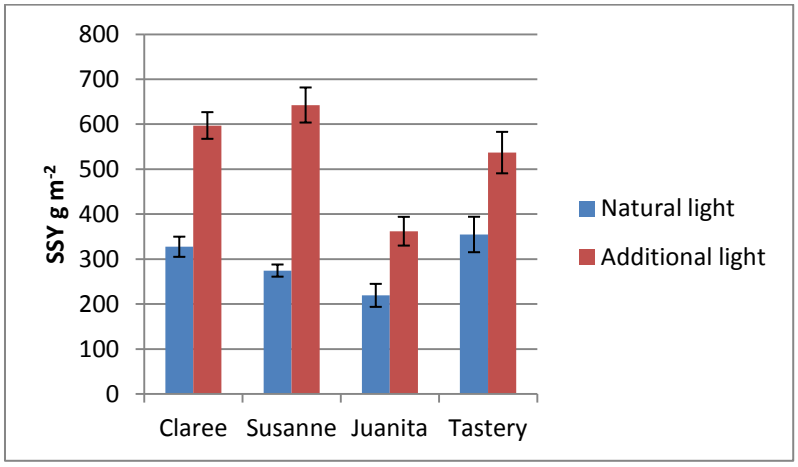


Figure 19. The influence of additional light on the soluble solids yield per m^2 for the total yield of four cherry tomato varieties.

Main effects and interactions of harvesting weeks, light, type and treatment on soluble solid yield per m^2 (SSY g m^{-2}) were also measured (Table 3, page 13). The different treatments did not affect SSY / m^2 considerably but it became a bit lower when the lowest nitrogen concentration was used (Fig. 19).

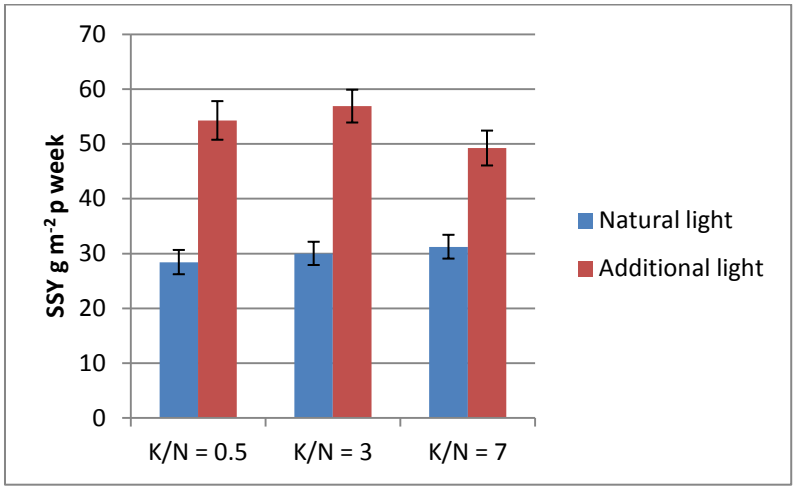


Figure 19. Soluble solids yield m-^{2} per week in cherry tomato varieties treated with three different nutrient solutions and grown under two light conditions.

The soluble solids yield m^{-2} per week was influenced differently in the four tomato varieties as can be seen in figure 20. The biggest difference was seen in Juanita, where lower K/N = 3 led to higher SSY / m^2.

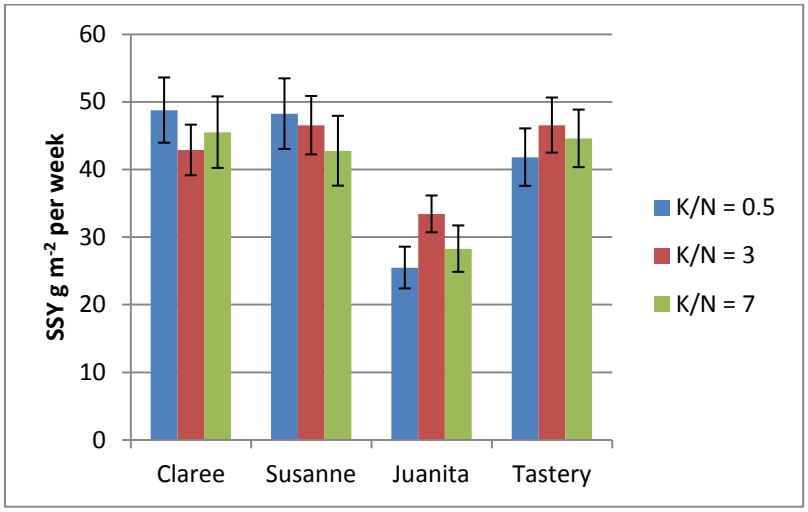
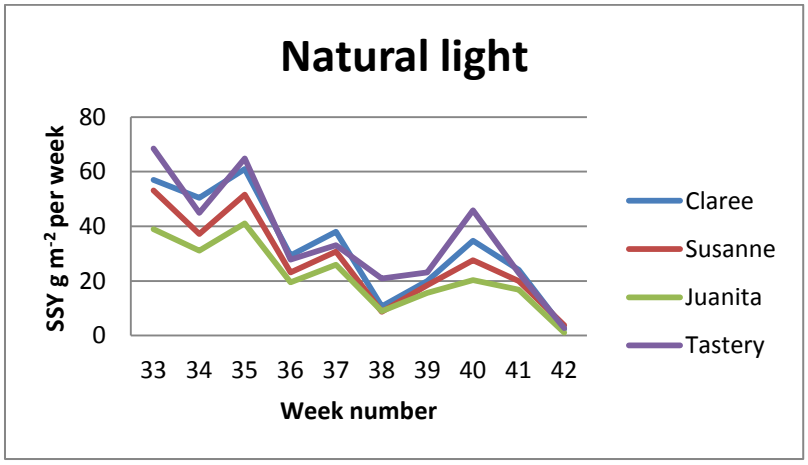


Figure 20. Soluble solids yield m^{-2} per week in four cherry tomato varieties treated with different nutrient solutions containing three different concentrations of nitrogen.

As illustrated in figure 21, the soluble solids per m^2 became lower in all tomato varieties during the harvesting weeks. Higher SSY/m^2 was found in the compartment where the plants were supplemented with extra light and in that compartment a bigger difference could be seen between the varieties. Juanita had the lowest SSY / m^2 in the study period (Fig. 21 a) and b)).

(A)



(B)

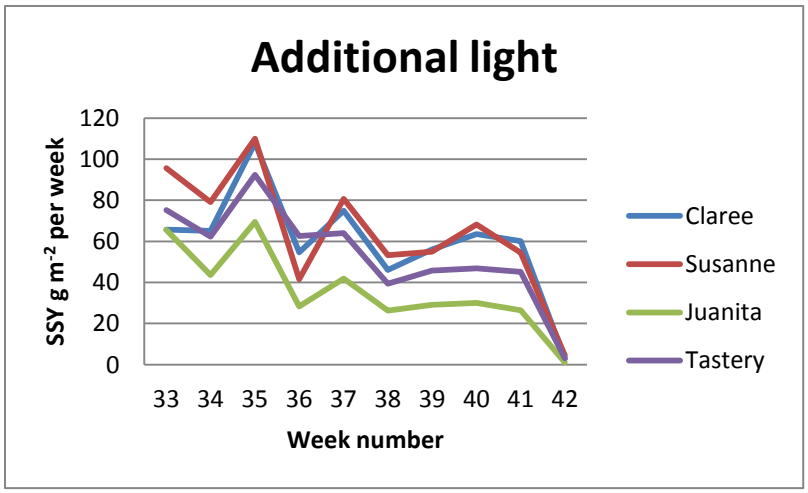


Figure 21. Soluble solids yield m^{-2} per week in four cherry tomatoes grown in natural light and supplemented with extra light. (A) Plants grown under natural light conditions. (B) Plants grown supplemented with additional light (240 µmol $m^{-2}s^{-1}$ photosynthetic active radiation, 18h/day when global radiation outside < 275 W/m^2).

4 Discussion

When plants are grown in greenhouses or in the field, different parameters affect their growth and their quality and must therefore be examined together. These factors can be climate factors like light and temperature. Nutrition can also influence the plant growth and quality.

Nitrogen content in the nutrient solution had little effect on SSC content in the tomato fruits. This was unexpected because it has been observed earlier at Bioforsk Vest Særheim that sugar content and acid concentrations in tomato fruits changed significantly five weeks after an alteration in nutrient composition. The differences between the present and earlier studies might be explained by differences in the outside global radiation. Earlier experiments were performed in winter to spring, when the outside global radiation was increasing during the growing season. In the present study outside global radiation decreased throughout the growing season from late summer to winter. A decreasing global radiation might have reduced expected increases in SSC contents due to reduced nitrogen content in the nutrient solution. Different amounts of nitrogen in the fertilizers had comparable effects in both growth environments. The present results confirm the existence of complex interactions of growth conditions on fruit quality. Another factor that must be taken into account is how accurately the samples must be picked due to growing stage. If the tomatoes are to green or to near the ripening stage, different ratio between sugars and acids might occur. Cherry tomatoes are especially sensitive to this. When picked near the ripening stage a higher sugar concentration is usually observed (Verheul, 2012).

The influence of different treatments interacted often with the other parameters. But when looked at more closely, it could be seen that different nitrogen levels in the fertilizers did not have considerable effects on the fruits. There may have been a negative interaction between the treatments and the light effect, where low radiation might have diminished the treatment effects. It was noticed though that the plants responded often positively to a high K/N ratio (low amount of nitrogen) and the biggest effects were seen in the fruits taste. High amount of nitrogen led to poor taste (except for ‘Claree’ and ‘Susanne’) and this supports the finding of Locascio et al. (1994) which reported that a high amount of nitrogen in the nutrition can lead to fruits with poor taste. It must be noticed though that there was an interaction between treatment and light and same cultivar responded differently to the same treatment under different light conditions. ‘Susanne’ had for example a better taste when grown with K/N = 7 in natural light but when supplemented with additional light a higher amount of nitrogen (K/N = 0.5) had the best effects. It has been found in

earlier research at Bioforsk Vest, Særheim that 'Susanne' produces fruits with better taste when grown with low amount of nitrogen in natural light (Verheul, unpublished data).

It is preferable to use a low amount of nitrogen in nutrient solution because of the negative influence nitrogen has on the environment. If too much nitrogen is added to fertilizers some of it might not be used by the plants causing it to end up in groundwater and drinking water. It is also positive for the producers to be able to reduce the amount of nitrogen in the fertilizer and thereby lowering the cost of nutrients. Earlier studies have shown that low nitrogen concentration in nutrients enhances production of phenolics in tomato plants (Løvdal et al. 2008; Larbat et al. 2011). Current study illustrated no negative impact of low nitrogen level on the quality of the fruits, recommending the use of lower content of nitrogen.

Different light conditions did not have considerable effects on SSC but the type effects were clearer and might be more important. It was expected to see a bigger difference between the two greenhouse compartments but it is possible that the artificial light did not supplement enough radiation for the tomatoes to produce more sugars when the global radiation became low. The reason could also be that supplemented light does not affect the SSC concentration in tomatoes considerably as Gautier (2008) and his coworkers concluded in their research.

The acid concentration fluctuated during the observation but the final concentration was similar to the initial one. Light seems to play a bigger role here since it is involved in all interaction effects. The results were most likely affected by the low radiation outside the greenhouse compartments, especially where the plants did not receive additional light. The acid concentration was a bit higher in plants grown without additional light, indicating an influence of lower global radiation and temperature. Gautier (2008) reported that temperature and irradiance do not affect the final concentration of acids which is in contrast to what was observed in current study. Gautier and coworkers analyzed off-vine ripening tomatoes during spring which could explain the difference in results.

Slimestad (2004) stated that SSC declined during early autumn and became stable in late autumn due to a high level of electrical conductivity (EC) in the growing medium. The EC was constant in current study but the medium might have kept the SSC concentration stable. It might be positive to increase EC during late autumn to achieve higher SSC concentration in the fruits.

Differences between cultivars were frequently observed and were more apparently than other parameters tested in this study. The weight was dissimilar, where Tastery produced the largest fruits while Juanita bore the lightest fruits. The variance could also be seen in taste, Susanne evaluated

with the best taste. The fruit firmness was stable through the growing season which was unexpected since it has been observed in earlier studies at Bioforsk Særheim that fruit firmness increased during the growing season (Verheul, unpublished data). Here, the main variation in firmness was found between cultivars. To our knowledge, these cultivars have never been compared before therefore giving interesting results.

Plants supplemented with additional light gave more yield than plants grown in natural light. The same was seen for fruit soluble solids per m^2. The fruit weight was similar in both greenhouse compartments but plants receiving extra light produced more fruits. This supports earlier findings of increased tomato crop with supplemental light (Cockshull et al. 1988; Verheul, 2012). These results could be one of the explanations of the stability of SSC in the fruits. When the plants are grown in additional light they might not use the extra energy to increase the sugar rate but to produce more fruits. When the plants were grown in natural light, they produced less fruit and the sugar concentration was similar. It was noticed that fruit weight for total yield was higher than for tomatoes picked for analysis. The reason could be that tomatoes that were harvested for analysis were usually located in the middle of the tomato cluster. These tomatoes are often smaller than fruits higher on the cluster which are near ripening stage. When yield is estimated, all tomatoes near or at ripening stage are picked so larger tomatoes are included in the sample influencing the final weight.

During the cultivation period, the yield became lower and the tomatoes got lighter in weight in both growth compartments. Again, the low global radiation might be the explanation, especially in the compartment with natural light where the radiation and the temperature became lower.

The yield was similar between the cultivars except for 'Juanita' which produced many tomatoes with low fruit weight. Different treatments did not have significant influence on the yield which was unexpected. Earlier findings have shown that low nitrogen concentration reduced the fruit size and the yield because of lower amount of photo assimilates available for the fruits (Dorais et al. 2001b). This was not confirmed in this research. This is positive because it is preferable to produce high yield while using less amount of nitrogen with respect to the environment.

Soluble solids yield per m^2 decreased during the harvesting weeks in both growth compartments. Higher SSY was observed when the plants were supplemented with additional light. "Juanita" had the lowest SSY per m^2 indicating its lower marketable value in this study. The other cultivars responded differently when grown with or without additional light. Different treatments had various effects on the SSY per m^2 in the tomatoes and the difference was more apparent than when SSC and yield were

analyzed separately. Higher SSY per m^2 was found when lower amount of nitrogen was used recommending modest use of this compound in nutrient solutions.

In this study, a lot of information have been gathered which can be used by tomato producers to improve the quality of their fruits. The main factors have been approached but more information lies within the data. The producer can analyze the data in more details for the cultivar they are growing to improve the conditions in their greenhouses. It would be desirable to look more closely at the SSC contents and titratable acidity to gain a better understanding on why these compounds were not more affected by the factors tested in present study. The experiment could be repeated to see if the same results are gained. Plant length should be included in the results to evaluate the influence of nitrogen and light on plant growth. The plant length was measured in current study, but the data have not been analyzed. It might also be interesting to look more closely at management practices like plant density and leaf/fruit ratio. Different tomato cultivars could be included in the research to see if other varieties are more desirable in this time of the year.

5 Conclusion

The results of this study confirm the existence of complex interactions of growth conditions on fruit quality. Various nitrogen levels in the nutrition solution had no considerable effects on the quality and yield of the tomato fruits, but it was noticed that fruits responded often positive to lower amount of nitrogen, recommending moderate use nitrogen in the nutrient solution. Different light condition had little effect on the fruit quality, but a higher yield was gained when the plants were supplemented with extra light. Biggest variation was found between the cultivars giving the producers possibility to choose the tomato cultivar most suitable to their greenhouse conditions or improving their growing compartments.

6 References

Alfnes, F., Guttormsen, A (2010) “The Perfect Tomato. Consumer preferences and willingness to pay for quality characteristics.” Report IRIS.

Anthon, G. E., LeStrange, M., Barret, D.M. (2011). "Changes in pH, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes." J Sci Food Agric **91(7)**: 1175-1181**.**

Balibrea M.E., Martinez-Andujar, C., Cuartero, J., Bolarin, M.C., Pérez-Alfocea, F. (2006). “The high sugar content in wild Lycopersicon species and their hybrids with cultivars depends on sucrose import during ripening rather than on sucrose metabolism.” Funct. Plant Biol**.(33)**: 279 – 288.

Beckles, D.M., Hong, N., Stamova, L., Luengwilai, K. (2011). “Biochemical factors contributing to tomato fruit sugar content: a review.” Fruits**(67)**: 49 - 64.

Dorais, M., P. Athanasios, Gosselin, A. (2001a). “Influence of electric conductivity management on greenhouse tomato yield and fruit quality.” Agronomie**(21)**: 367-383.

Dorais, M., A.P. Papadopoulos, Gosselin, A. (2001b). “Greenhouse Tomato Fruit Quality.” Horticultural Reviews**(26)**: 239-319.

Clé, C., L.M. Hill, Niggeweg, RR, Martin, C.R., Guizes, Y., Prinsen, E., Jansen, M.A.K. (2008). ”Modulation of chlorogenic acid biosynthesis in *Solanum lycopersicu;* consequences for phenolic accumulation and UV-tolerance. Phytochemistry **69**: 2149-2156.

Cockshull, K. E. (1988). “The integration of plant physiology with physical changes in the greenhouse climate.” Acta Horticulturae **(229)**: 113 – 123.

Fujisawa, M., Shima,Y., Higuchi N., Nakano T., Koyama Y., Kasumi T., Ito Y. (2011). "Direct targets of the tomato-ripening regulator RIN identified by transcriptome and chromatin immunoprecipitation analyses." Planta.

Gautier, H., Diakou-Verdin V., Bénard, C., Reich, M., Buret, M., Bourgaud, F., Poëssel, J.L., Caris-Veyrat, C., Génard, M. (2008). “How does Toamto Quality (Sugar, Acid, and Nutritional Quality) Vary with Ripening Stage, Temperature, and Irradiance.” J Agric Food Chem **56(4)**: 1241 – 50.

Gillaspy, G., Ben-David, H., Gruissem, W. (1993). "Fruits: A Developmental Perspective." Plant Cell **5(10)**: 1439-1451.

Heuvelink, E., M. Dorais (2005). “Crop Growth and Yield.” P. 85 – 144. In E. Heuvlink (ed.), *Tomatoes*. *Crop Production Science in Horticulture 13*. CABU Publishing.

Klee, H. J. (2010). "Improving the flavor of fresh fruits: genomics, biochemistry, and biotechnology." New Phytol **187(1)**: 44-56**.**

Larbat, R., Olsen, K.M., Slimestad, R., Løvdal, T., Bénard, C., Verheul, M., Bourgaud, F., Robin, C., Lillo, C. (2011). ” Influence of repeated short-term nitrogen limitations on leaf phenolicsmetabolism in tomato.” Phytochemistry **77**: 119-128.

Lillo, C., Lea, U.S., Ruoff, P. (2008). "Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway." Plant Cell Environm. **31**(5): 587-601.

Locascio, S.J., Wiltbank, W.J., Gull, D.D., Maynard, D.N. (1984). "Fruit and vegetable quality as affected by nitrogen nutrition", In Nitrogen in crop production: 617 – 626

Luengwilai, K., Beckles, D.M. (2009). "Starch granules in tomato fruit show a complex pattern of degradation." J Agric Food Chem **57(18)**: 8480-8487.

Lovdal, T., Olsen, K.M., Slimestad, R., Verheul, M., Lillo, C. (2010). "Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato." Phytochemistry **71(5-6)**: 605-613.

Malundo TMM., Shewfelt, R.L., Scott, J.W. (1994). "Flavor quality of fresh tomato (*Lycopersicon esculentum* Mill.) as affected by sugar and acid levels." Postharvest Biology and Technology**(6)**: 103 – 110.

Pete, M. M. (2005). "Irrigation and Fertilization." P. 171 – 198. In E. Heuvlink (ed.), *Tomatoes. Crop Production Science in Horticulture 13*. CABI Publishing.

Slimestad, R., Verheul, M.J. (2005). "Seasonal variations in the level of plant constituents in greenhouse production of cherry tomatoes." J Agric Food Chem **53(8)**: 3114-3119.

Steinhauser, M. C., Steinhauser, D., Koehl, K., Carrari, F., Gibon, Y., Fernie, A.R., Stitt, M. (2010). "Enzyme activity profiles during fruit development in tomato cultivars and Solanum pennellii." Plant Physiol **153(1)**: 80-98.

Stevens, MA., Kader, A.A., Albright, M. (1979). "Potential for increasing Tomato Flavor via Increased Sugar and Acid Content." J Amer Soc Hort Sci **104(1)**: 40 – 42.

Verheul, M. J. (2003). "Sunne planter, sunnere kosthold. Virkningen av dyrkingsmedium på plantekvalitet og fruktkvalitet i agurk." Report Planteforsk Særheim forskningsenter.

Verheul, M. J. (2004). "Kartlegging av smaksegenskaper i tomat." Report Planteforsk Særheim.

Verheul, M.J. (2012). "Effects of plant density, leaf removal and light intensity on tomato quality and yield." Acta Horticulturae. ISHS Lightsym 2012 (in press).

Verheul, M.J., Slimestad, R., Holta Tjøstheim, I. (2012). "From producer to consumer- greenhouse tomato quality as affected by maturity stage at harvest, simulated transport and supermarket storage" (in preparation)

Wann, E. V. (1996). "Physical characteristics of mature green and ripe tomato fruit tissue of normal and firm genotypes." J Amer Soc Hort Sci **121(3)**: 380 - 383.

Yelle, S., Chetelat, R.T., Dorais, M., DeVerna, J.W., Bennet, A.B. (1991). "Sink Metabolism in Tomato Fruit : IV. Genetic and Biochemical Analysis of Sucrose Accumulation." Plant Physiol **95(4)**: 1026-1035.

Appendix A

Anthocyanin measurement

Experiment 1

Table A1. Absorbance of anthocyanins measured in spectrophotometer (AnalytikJena SPECORD 200) at 530 nm and 657 nm. Plants were grown on rock wool and treated with/without nitrogen for 5 days in continuous light.

Ecotype	Weight (g)	$Absorbance_{530}$	$Absorbance_{657}$	$Absorbance_{530}$ - $Absorbance_{657}$	Absorbance/g
Parallell 1					
WT WS+N	0.054	0.03	0.01	0.02	0,31
WT WS-N	0.050	0.47	0.06	0.33	6,50
***35 cpc*+N**	0.051	0.04	0.04	-0.006	-0,12
***35 cpc*-N**	0.050	0.28	0.04	0.24	4,84
***cpc – 1*+N**	0.052	0.07	0.03	0.03	0,66
***cpc – 1*-N**	0.050	0.59	0.02	0.57	11,40
WT Col+N	0.050	0.15	0.03	0.12	2,41
WT Col-N	0.051	0.53	0.03	0.51	9,90
***try* + N**	0.050	0.05	0.02	0.04	0,76
***try* - N**	0.050	0.86	0.02	0.83	16.00
***mybl2*+N**	0.050	0.07	0.02	0.05	1.07
***mybl2*- N**	0.050	0.65	0.03	0.62	12.38
Parallell 2					
WT WS+N	0.051	0.03	0.02	0.01	0.15
WT WS-N	0.051	0.42	0.02	0.40	7.73
***35 cpc*+N**	0.051	0.04	0.02	0.02	0.39
***35 cpc*-N**	0.050	0.50	0.04	0.46	9.13
***cpc – 1*+N**	0.051	0.04	0.03	0.01	0.36
***cpc – 1*-N**	0.050	0.72	0.009	0.71	14.25
WT Col-N	0.051	0.05	0.02	0.03	0.45
WT Col-N	0.051	0.64	0.02	0.62	12.20
***try*+N**	0.050	0.06	0.01	0.04	0.88
***try*–N**	0.050	0.79	0.03	0.76	15.11
***mybl2*+N**	0.051	0.11	0.02	0.096	1.89
***mybl2*-N**	0.050	0.72	0.005	0.72	14.34
Parallell 3					
WT WS+N	0.05	0.04	0.005	0.03	0.62
WT WS-N	0.05	0.46	0.005	0.46	9.09
***35 cpc*+N**	0.05	0.04	0.007	0.03	0.61
***35 cpc*-N**	0.05	0.56	0.007	0.55	11.08
***cpc – 1*+N**	0.05	0.05	0.01	0.04	0.69
***cpc – 1*-N**	0.046	0.40	0.006	0.39	8.56
WT Col+N	0.051	0.05	0.007	0.04	0.81
WT Col-N	0.051	0.74	0.007	0.73	14.28

***try*+N**	0.051	0.07	0.009	0.06	1.23
***try*-N**	0.042	0.94	0.01	0.92	21.99
***mybl2*+N**	0.051	0.14	0.007	0.14	2.65
***mybl2*-N**	0.050	0.81	0.009	0.80	15.93

Table A2. Absorbance of anthocyanins measured in spectrophotometer (AnalytikJena SPECORD 200) at 530 nm and 657 nm. Plants were grown on rock wool and treated without nitrogen for 10 days in cont. light and 5 days without nitrogen in 16 h / 8 h photoperiode.

Ecotype	**Weight (g)**	**Absorbance$_{530}$**	**Absorbance$_{657}$**	**Absorbance$_{530}$ - Absorbance$_{657}$**	**Absorbance/g**
Parallel 1					
WT WS-N	0.050	0.35	0.07	0.29	5.69
***35 cpc*-N**	0.050	0.31	0.01	0.30	5.92
***cpc – 1*-N**	0.051	0.81	0.02	0.78	15.35
WT Col-N	0.052	0.42	0.02	0.40	7.65
***try* - N**	0.050	0.47	0.02	0.45	8.98
***mybl2*-N**	0,050	0,61	0,008	0,60	11,95
Parallel 2					
WT WS-N	0.050	0.39	0.02	0.37	7.29
***35 cpc*-N**	0.051	0.23	0.016	0.21	4.17
***cpc – 1*-N**	0.050	0.40	0.006	0.40	7.95
WT Col-N	0.051	0.52	0.02	0.50	9.92
***try*-N**	0.050	0.7	0.02	0.68	13.65
***mybl2*-N**	0.051	0.42	0.008	0.41	8.13
Parallel 3					
WT WS-N	0.051	0.26	0.01	0.25	4.81
***35 cpc*-N**	0.050	0.50	0.02	0.48	9.64
***cpc – 1*-N**	0.051	0.65	0.008	0.64	12.56
WT Col-N	0.051	0.23	0.008	0.22	4.38
***try*–N**	0.051	0.69	0.02	0.67	13.21
***mybl2*-N**	0.050	0.64	0.008	0.63	12.52

Experiment 2

Table A3. Absorbance of anthocyanins measured in spectrophotometer (AnalytikJena SPECORD 200) at 530 nm and 657 nm. Plants grown in continuous light and treated with and without nitrogen for 5 days.

Ecotype	Weight (g	$Absorbance_5$	$Absorbance_6$	$Absorbance_{530} - Absorbance_6$	Absorbance/
Parallel 1					
WT WS+N	0.049	0.01	-0.003	0.02	0.35
WT WS–N	0.051	0.15	0.01	0.14	2.72
***35S cpc*+N**	0.051	0.02	0.008	0.01	0.24
***35S cpc*-N**	0.051	0.07	0.002	0.07	1.42
***cpc-1*+N**	0.051	0.03	0.02	0.01	0.20
***cpc -1*-N**	0,051	0.06	0.01	0.04	0.84
WT Col+N	0.050	0.05	0.01	0.04	0.69
WT Col–N	0.051	0.54	0.01	0.53	10.43
***try*+N**	0.050	0.05	0.01	0.04	0.80
***try*-N**	0.051	0.57	0.001	0.56	10.97
***mybl2*+N**	0.052	0.05	0.009	0.04	0.85
***mybl2*-N**	0.050	0.1	0	0.1	19.88
Parallel 2					
WT WS+N	0.050	0.03	0.01	0.02	0.38
WT WS–N	0.050	0.10	0.002	0.10	2.03
***35S cpc*+N**	0.050	0.02	0.02	0.005	0.10
***35S cpc*-N**	0.051	0.08	0.006	0.08	1.49
***cpc-1*+N**	0.050	0.05	0.02	0.02	0.48
***cpc -1*-N**	0.051	0.13	0.009	0.12	2.39
WT Col+N	0.050	0.05	0.02	0.04	0.78
WT Col–N	0.050	0.26	0.01	0.24	4.90
***try*+N**	0.051	0.04	0.003	0.035	0.69
***try*-N**	0.046	0.27	-0.001	0.27	5.88
***mybl2*+N**	0.052	0.03	0.06	-0.02	-0.44
***mybl2*-N**	0.050	0.90	0.03	0.87	17.39
Parallel 3					
WT WS+N	0.050	0.02	0.002	0.02	0.32
WT WS–N	0.050	0.32	0.009	0.31	6.21
***35S cpc*+N**	0.050	0.02	0.02	0.003	0.06
***35S cpc*-N**	0.050	0.06	0.01	0.06	1.14
***cpc-1*+N**	0.050	0.03	0.01	0.02	0.42
***cpc -1*-N**	0.051	0.22	0.02	0.20	3,90
WT Col+N	0.050	0.05	0.001	0.05	0.96
WT Col–N	0.050	0.24	0.02	0.23	4.59
***try*+N**	0.051	0.04	0.02	0.02	0.38
***try*-N**	0.051	0.27	0.01	0.25	4.96

***mybl2*+N**	0.050	0.03	0.007	0.03	0.50
***mybl2*-N**	0.050	1.05	0.02	1.03	20.72

Table A4. Absorbance of anthocyanins measured in spectrophotometer (AnalytikJena SPECORD 200) at 530 nm and 657 nm. Plants were grown in 16 h /8 h growth chamber and treated with and without nitrogen for 7 days.

Ecotype	Weight (g	$Absorbance_5$	$Absorbance_6$	$Absorbance_{530} - Absorbance_6$	Absorbance/
Parallel 1					
WT WS+N	0.051	0.008	0.004	0.004	0.07
WT WS–N	0.051	0.36	0.006	0.35	6.88
***35S cpc*+N**	0.052	0.006	0.002	0.004	0.08
***35S cpc*-N**	0.052	0.43	0.007	0.42	8.19
***cpc-1*+N**	0.051	0.01	0.003	0.008	0.16
***cpc -1*-N**	0.052	0.62	0.01	0.61	11.72
WT Col+N	0.049	0.01	0.003	0.01	0.22
WT Col–N	0.050	0.61	0.005	0.60	12.07
***try*+N**	0.052	0.008	0.004	0.004	0.08
***try*-N**	0.050	0.50	0.003	0.50	9.92
***Mybl2*+N**	0.051	0.01	0.009	0.002	0.03
***mybl2*-N**	0.050	0.70	0.006	0.69	13.72
Parallel 2					
WT WS+N	0.051	0.02	-0.001	0.02	0.39
WT WS–N	0.050	0.45	-0.01	0.46	9.21
***35S cpc*+N**	0.050	0.04	-0.003	0.05	0.9
***35S cpc*-N**	0.050	0.41	-0.02	0.42	8.42
***cpc-1*+N**	0.050	0.04	0.02	0.03	0.52
***cpc -1*-N**	0.050	0.65	0.002	0.65	12.83
WT Col+N	0.050	0.07	-0.004	0.07	1.48
WT Col–N	0.051	0.41	-0.007	0.42	8.2
***try*+N**	0.050	0.02	-0.008	0.03	0.50
***try*-N**	0.051	0.40	-0.001	0.40	7.98
***mybl2*+N**	0.050	0.02	0.007	0.01	0.20
***mybl2*-N**	0.050	0.34	-0.03	0.37	7.39
Parallel 3					
WT WS+N	0.050	0.03	0.01	0.02	0.30
WT WS–N	0.050	0.46	0.01	0.45	8.98
***35S cpc*+N**	0.050	0.04	0.03	0.008	0.16
***35S cpc*-N**	0.050	0.43	0.02	0.41	8.26
***cpc-1*+N**	0.050	0.03	0.008	0.03	0.52
***cpc -1*-N**	0.051	0.40	0.002	0.40	7.8
WT Col+N	0.050	0.02	0.004	0.01	0.22
WT Col–N	0.050	0.49	0.003	0.49	9.73
***try*+N**	0.050	0.02	-0.003	0.02	0.40
***try*-N**	0.050	0.33	0.005	0.32	6.49
***mybl2*+N**	0.050	0.03	-0.02	0.04	0.88
***mybl2*-N**	0.050	0.39	0.02	0.37	7.37

Experiment 3

Table A5. Absorbance of anthocyanins measured in spectrophotometer (AnalytikJena SPECORD 200) at 530 nm and 657 nm. The plants were treated without nitrogen for 5 days in continuous light.

Ecotype	Weight (g	$Absorbance_5$	$Absorbance_{657}$	$Absorbance_{5}$ - $Absorbance_{6}$	Absorbance/g
Parallel 1					
WT WS+N	0.052	0	0.01	0.01	0.19
WT WS-N	0.050	0.02	0.32	0.34	6.37
35S cpc+N	0.050	-0.005	0.01	0.009	0.28
35S cpc-N	0.054	-0.004	0.64	0.63	11.88
cpc-1+N	0.052	0.008	0.02	0.03	0.42
cpc-1-N	0.050	0.005	0.57	0.57	11.31
WT col+N	0.051	0.01	0.06	0.08	1.25
WT Col-N	0.050	0.004	0.73	0.73	14.70
try+N	0.052	-0.02	0.03	0.01	0.65
try-N	0.049	-0.02	0.64	0.62	12.99
mybl2+N	0.050	-0.02	0.04	0.02	0.70
mybl2-N	0.050	-0.02	1.05	1.04	21.10
Parallell 2					
WT WS+N	0.050	0.008	0.01	0.02	0.24
WT WS-N	0.050	-0.007	0.33	0.33	6.71
35S cpc+N	0.051	0.004	0.03	0.03	0.58
35S cpc-N	0.053	0.009	0.55	0.55	10.38
cpc-1+N	0.051	0.01	0.02	0.03	0.37
cpc-1-N	0.049	0.007	0.52	0.53	10.51
WT col+N	0.050	0.005	0.04	0.04	0.71
WT Col-N	0.050	0.002	0.98	0.98	19.38
try+N	0.050	-0.02	0.05	0.02	0.96
try-N	0.050	-0.03	0.75	0.72	14.88
mybl2+N	0.050	-0.02	0.05	0.03	0.92
mybl2-N	0.052	-0.006	1.14	1.13	22.00
Parallel 3					
WT WS+N	0.051	0.004	0.01	0.01	0.20
WT WS-N	0.050	0.01	0.55	0.56	11.08
35S cpc+N	0.051	0.001	0.03	0.03	0.51
35S cpc-N	0.051	0.01	0.59	0.60	11.42
cpc-1+N	0.051	0.002	0.02	0.03	0.46
cpc-1-N	0.050	-0.005	0.74	0.73	14.83
WT col+N	0.050	-0.006	0.04	0.04	0.86
WT Col-N	0.051	0.07	0.50	0.56	9.86
try+N	0.050	-0.006	0.03	0.02	0.54
try-N	0.051	-0.01	0.71	0.69	13.76
mybl2+N	0.051	-0.02	0.05	0.03	0.89
mybl2-N	0.051	-0.006	1.04	1.03	20.43

Combined measurements

Table A6. Absorbance of anthocyanins per gram, measured in spectrophotometer (AnalytikJena SPECORD 200). Standard deviation and standard error are also shown.The data are average absorbance of three combined experiments (see tables A1, A3 and A5). Plants were grown in continuous light.

Ecotye	Absorbance per gram. average	Standard deviation	Standard error
WT WS + N	0.31	0.10	0.07
WT WS - N	6.49	2.46	1.74
***35S cpc* + N**	0.29	0.12	0.08
***35S cpc* - N**	6.98	5.08	3.59
***cpc - 1* + N**	0.45	0.12	0.08
***cpc - 1* - N**	8.67	5.46	3.86
WT COL + N	1.02	0.26	0.15
WT COL - N	10.92	4.03	2.33
***try* + N**	0.77	0.17	0.01
***try* - N**	12.95	5.28	3.05
***mybl2* + N**	1.002	0.80	0.46
***mybl2* - N**	18.24	3.61	2.082

Table A7. Absorbance of anthocyanins per gram, measured in spectrophotometer (AnalytikJena SPECORD 200). Standard deviation and standard error are also shown.The data are average absorbance of two combined experiments for –N and one experiment for + N (see tables A2 and A4). Plants were grown in 16 h light/8 h dark rhythm.

Ecotype	Absorbance per gram. average	Standard deviation	Standard error
WT WS + N	0.25		0
WT WS - N	7.14	1.72	1.22
***35S cpc* + N**	0.38		0
***35S cpc* - N**	7.43	1.21	0.86
***cpc - 1* + N**	0.4		0
***cpc - 1* - N**	11.37	0.83	0.59
WT COL + N	0.64		0
WT COL - N	9.54	0.66	0.46
***try* + N**	0.33		0
***try* - N**	10.04	2.70	1.91
***mybl2* + N**	0.37		0
***mybl2* - N**	11.86	3.34	2.36

Relative concentration

Table A8. Absorbance of anthocyanins per gram, measured in spectrophotometer (AnalytikJena SPECORD 200). Standard deviation and standard error are also shown.The data show relative anthocyanin con. in three combined experiments for –N and two experiment for + N (see tables 8, 10 and 12). Plants were grown in continuous light and treated for 5 days.

Ecotype	Relative anthocyanin con.	Standard deviation	Standard error
WT WS + N	6,5	5,0	2,9
WT WS - N	100,0	0,0	0,0
***35S cpc* + N**	4,7	1,3	0,8
***35S cpc* - N**	100,3	50,2	29,0
***cpc - 1* + N**	8,5	3,6	2,1
***cpc - 1* - N**	126,0	48,1	27,8
	11,0	3,8	2,2
WT COL + N	100,0	0,0	0,0
WT COL - N	7,6	2,5	1,5
***try* + N**	119,7	23,7	13,7
***try* - N**	9,3	8,0	4,6
***mybl2* + N**	207,9	108,2	62,5
***mybl2* - N**	6,5	5,0	2,9

Table A9. Absorbance of anthocyanins per gram, measured in spectrophotometer (AnalytikJena SPECORD 200). Standard deviation and standard error are also shown.The data show relative anthocyanin con. in two combined experiments for –N and one experiment for + N (see tables 8, 10 and 12). Plants were grown in 16 h light/8 h dark rhythm.

Ecotype	Relative anthocyanin con.	Standard deviation	Standard error
WT WS + N	2,9		0
WT WS - N	100,0	0	0
***35S cpc* + N**	4,2		0
***35S cpc* - N**	110,7	14,0	9,9
***cpc - 1* + N**	4,6		0
***cpc - 1* - N**	172,7	57,4	40,6
	7,4		0
WT COL + N	100,0	0	0
WT COL - N	3,6		0
***try* + N**	109,3	38,5	27,3
***try* - N**	3,9		0
***mybl2* + N**	108,6	21,8	15,4
***mybl2* - N**	2,9		0

Gene expression

Seedling stage

Table A10. Quality of RNA measured by Nanodrop. Plants treated with and without nitrogen for 7 days in continuous light. Nucleic acid concentration was found by multiplying A260 and Factor.

#	Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	Wt+N	762.8	ng/µl	19.070	8.843	2.16	2.11	RNA	40.00
2	Egl3+N	1417.5	ng/µl	35.437	16.343	2.17	2.36	RNA	40.00
3	Gl3+N	1047.9	ng/µl	26.197	12.162	2.15	2.42	RNA	40.00
4	Wt-N	78.8	ng/µl	1.970	0.873	2.26	0.17	RNA	40.00
5	Egl3-N	197.5	ng/µl	4.938	2.249	2.20	0.86	RNA	40.00
6	Gl3-N	119.5	ng/µl	2.988	1.359	2.20	0.28	RNA	40.00

Table A11. Avg Cq values for *ACT8, DFR, CPC, MYBL2* and *TRY*. Plants were grown on Petri dishes and treated for 7 days in continuous light.

Sample	Avg Cq *ACT8*	Avg Cq *Ubq*	Avg Cq *DFR*	Avg Cq *CPC*	Avg Cq *MYBL2*	Avg Cq *TRY*
WT-Ler +N	25.432	25.276	29.799	30.292	23.811	31.364
WT-Ler -N	26.307	26.893	22.531	29.596	24.455	34.303
egl3 +N	27.061	27.051	29.611	31.913	24.460	30.192
***egl3* -N**	25.907	26.565	23.355	28.439	26.451	32.223
***gl3* +N**	26.794	26.310	33.715	32.305	21.611	31.420
***gl3* -N**	25.609	25.745	30.194	29.492	27.483	32.262

Table A12. Avg RQ values for *DFR, CPC, MYBL2* and *TRY*. Plants were grown on Petri dishes and treated for 7 days in continuous light.

Sample	Avg RQ *DFR*	Avg RQ *CPC*	Avg RQ *MYBL2*	Avg RQ *TRY*
WT-Ler +N	1	1	1	1
WT-Ler -N	283	2,97	1,38	0,24
egl3 +N	3,52	1,01	1,97	6,97
***egl3* -N**	121	5,02	0,22	0,77
***gl3* +N**	0,17	0,64	11,8	2,47
***gl3* -N**	0,86	1,97	0,09	0,61

Rosette stage

Table A13. Quality of RNA measured by Nanodrop. Plants treated with and without nitrogen for 3 days in 16 h light/ 8 h dark rhythm. Nucleic acid concentration was found by multiplying A260 and Factor.

#	Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	Wt+N	877.0	ng/µl	21.926	9.942	2.21	2.22	RNA	40.00
2	Egl3+N	318.3	ng/µl	7.958	3.648	2.18	1.32	RNA	40.00
3	Gl3+N	1283.1	ng/µl	32.077	14.619	2.19	2.40	RNA	40.00
4	Wt-N	326.7	ng/µl	8.168	3.763	2.17	1.32	RNA	40.00
5	Egl3-N	1008.0	ng/µl	25.201	11.422	2.21	1.83	RNA	40.00
6	Gl3-N	429.5	ng/µl	10.738	4.952	2.17	1.90	RNA	40.00

Table A14. Avg. Cq values for *ACT8, DFR, CPC, MYBL2* and *TRY*. Plants were treated for 3 days in 16 h light/ 8 h dark rhythm .

Sample	Avg Cq *ACT8*	Avg Cq *DFR*	Avg Cq *CPC*	Avg Cq *MYBL2*	Avg Cq *TRY*
WT-Ler +N	26.586	26.418	30.942	23.952	29.086
WT-Ler -N	25.992	23.153	27.564	23.528	28.995
egl3 +N	25.887	28.393	31.064	23.764	30.001
***egl3* -N**	26.121	23.599	27.780	23.999	29.044
***gl3* +N**	26.629	32.052	32.483	23.460	29.737
***gl3* -N**	26.411	29.895	31.004	23.890	29.023

Table A15. Avg. RQ values for *DFR, CPC, MYBL2* and *TRY*. Plants were grown on rock wool and treated for 3 days in 16 h light/ 8 h dark rhythm.

Sample	Avg RQ *DFR*	Avg RQ *CPC*	Avg RQ *MYBL2*	Avg RQ *TRY*
WT-Ler +N	1	1	1	1
WT-Ler -N	6.4	6.8	0.9	0.7
egl3 +N	0.2	0.6	0.7	0.3
***egl3* -N**	5.1	6.5	0.7	0.8
***gl3* +N**	0.02	0.4	1.5	0.7
***gl3* -N**	0.08	0.9	0.9	0.9

Table A16. Avg. Cq values for *ACT8, DFR, CPC, MYBL2* and *TRY*. Plants were treated for 5 days in 16 h light/ 8 h dark rhythm.

Sample	Avg Cq *Ubq*	Avg Cq *DFR*	Avg Cq *CPC*	Avg Cq *MYBL2*	Avg Cq *TRY*
WT-Ler +N	26.915	26.452	30.124	23.932	28.332
WT-Ler -N	26.583	22.399	27.808	23.384	28.232
egl3 +N	27.636	31.343	31.909	24.432	29.827
***egl3* -N**	27.316	24.337	28.738	24.121	29.862
***gl3* +N**	27.902	34.128	32.502	24.475	29.431
***gl3* -N**	27.376	27.822	29.938	25.082	29.552

Table A17. Avg. RQ values for *DFR, CPC, MYBL2* and TRY. Plants were grown on rock wool and treated for 5 days in 16 h light/ 8 h dark rhythm.

Sample	Avg RQ *DFR*	Avg RQ *CPC*	Avg RQ *MYBL2*	Avg RQ *TRY*
WT-Ler +N	1	1	1	1
WT-Ler -N	13,18	3,953	1,161	0,851
egl3 +N	0,056	0,478	1,165	0,585
***egl3* -N**	5,723	3,449	1,159	0,457
***gl3* +N**	0,01	0,381	1,36	0,925
***gl3* -N**	0,533	1,566	0,62	0,591

WT WS and WT COL

Table A18. Quality of RNA measured by Nanodrop. Plants grown in continuous light and treated with and without nitrogen for 5 days. Nucleic acid concentration was found by multiplying A260 and Factor.

#	Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	Wt WS +N	429.5	ng/µl	10.739	4.973	2.16	2.16	RNA	40.00
2	WT WS -N	54.3	ng/µl	1.359	0.645	2.11	0.25	RNA	40.00
3	WT COL +N	505.4	ng/µl	12.635	5.700	2.22	2.29	RNA	40.00
4	WT COL -N	75.8	ng/µl	1.895	0.887	2.14	1,17	RNA	40.00

Table A19. Quality of RNA measured by Nanodrop. Plants grown in 16 h day / 8 g night rytm and treated with and without nitrogen for 7 days. Nucleic acid concentration was found by multiplying A260 and Factor.

#	Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	WT WS +N	413.8	ng/µl	10.345	4.729	2.19	1.80	RNA	40.00
2	WT WS -N	77.5	ng/µl	1.938	0.864	2.24	0.13	RNA	40.00
3	WT COL + N	622.6	ng/µl	15.564	6.882	2.26	1.52	RNA	40.00
4	WT COL - N	138.3	ng/µl	3.457	1.593	2.17	1.84	RNA	40.00

Table A20. Rosette stage. Avg. Cq values for *ACT8, CPL3, CPC, DFR, ETC1, ETC2, MYBL2, TCL1, TRY* and *Ubq* in WT WS and WT Col. The plants where grown in continuous light and were treated with and without nitrogen for 5 days. *ACT8* and *Ubq* were used as endogenous controls.

Sample	*ACT8*	*CPC*	*CPL3*	*DFR*	*ETC1*	*ETC2*	*MYBL2*	*TCL1*	*TRY*	*Ubq*
***ACT8* used as endogenous control**										
WT WS + N	24.16	29.43	29.76	26.45	30.93	31.70	23.95	31.39	29.01	25.96
WT Col - N	25.10	28.81	25.10	21.98	31.9	34.8	23.78	34.9	29.16	25.79
***Ubq* used as endogenous control**										
WT WS + N	24.16	29.43	29.76	26.45	30.93	31.70	23.95	31.39	29.01	25.96
WT WS - N	25.10	28.81	27.32	21.98	31.9	34.8	23.78	34.9	29.16	25.79
***ACT8* used as endogenous control**										
WT Col + N	23.4	29.83	29.5	28.51	31.3	31.63	25.32	31.73	28.92	25.57
WT Col - N	25.14	30.29	27.93	23.71	31.82	34.41	26.1	34.74	31.43	26.88
***Ubq* used as endogenous control**										
WT Col + N	23.4	29.83	29.5	28.51	31.29	31.63	25.32	31.73	28.92	25.57
WT Col - N	25.14	30.29	27.93	23.71	31.82	34.41	26.1	34.74	31.43	26.88

Table A21. Rosette stage. Avg. RQ values for *CPC, CPL3, DFR, ETC1, ETC2, MYBL2, TCL1* and *TRY* in WT WS and WT Col. The plants where grown in continuous light and were treated with and without nitrogen for 5 days. *ACT8* and *Ubq* were used as endogenous controls.

Sample	*CPC*	*CPL3*	*DFR*	*ETC1*	*ETC2*	*MYBL2*	*TCL1*	*TRY*
***ACT8* used as endogenous control**								
WT WS + N	1	1	1	1	1	1	1	1
WT WS - N	2.9	10.4	42.7	0.9	0.2	2.2	0.2	1.8
***Ubq* used as endogenous control**								
WT WS + N	1	1	1	1	1	1	1	1
WT WS - N	1.4	4.9	19.8	0.5	0.1	1.0	0.08	0.8
***ACT8* used as endogenous control**								
WT Col + N	1	1	1	1	1	1	1	1
WT Col - N	2.4	9.9	92.5	2.3	0.5	1.9	0.4	0.6
***Ubq* used as endogenous control**								
WT Col + N	1	1	1	1	1	1	1	1
WT Col - N	1.8	7.4	68.8	1.7	0.4	1.4	0.3	0.4

Table A22. Rosette stage. Avg. Cq values for *ACT8, CPL3, CPC, DFR, ETC1, ETC2, MYBL2, TCL1, TRY* and *Ubq* in WT WS and WT Col. The plants where grown in 16 h day/8 h night rhythm and were treated with and without nitrogen for 7 days. *ACT8* and *Ubq* was used as endogenous control.

Sample	*ACT8*	*CPC*	*CPL3*	*DFR*	*ETC1*	*ETC2*	*MYBL2*	*TCL1*	*TRY*	*Ubq*
***ACT8* as endogenous control**										
WT WS + N	24.6	30.6	27.9	24.9	32.1	32.1	24.5	32.3	28.6	26.0
WT WS – N	23.7	28.2	24.8	21.0	29.7	30.8	23.0	31.8	28.2	25.0
***Ubq* as endogenous control**										
WT WS + N	24.62	30.6	27.85	24.86	32.06	32.11	24.49	32.33	28.6	25.95
WT WS – N	23.78	28.16	24.81	21.02	29.71	30.79	23.03	31.80	28.17	25.01
***ACT8* used as endogenous control**										
WT Col + N	23.37	29.97	29.31	27.94	31.53	31.18	24.4	32.24	27.92	25.86
WT Col -N	23.96	28.84	25.68	21.86	30.93	3.98	21.98	32.57	28.82	25.41
***Ubq* used as endogenous control**										
WT Col + N	23.37	29.97	29.31	27.94	31.53	31.18	24.4	32.24	27.92	25.86
WT Col - N	23.96	28.84	25.68	21.86	30.93	32.98	21.98	32.57	28.82	25.41

Table A23. Rosette stage. Avg. RQ values for *CPL3, CPC, DFR, ETC1, ETC2, MYBL2, TCL1* and *TRY* in WT WS and WT Col. The plants where grown in 16 h day/8 h night rhythm and were treated with and without nitrogen for 7 days. *ACT8* and *Ubq* were used as endogenous controls.

Sample	*CPC*	*CPL3*	*DFR*	*ETC1*	*ETC2*	*MYBL2*	*TCL1*	*TRY*
***ACT8* used as endogenous control**								
WT WS + N	1	1	1	1	1	1	1	1
WT Col - N	3.03	4.6	7.9	2.8	1.4	1.6	0.8	0.8
***Ubq* used as endogenous control**								
WT WS + N	1	1	1	1	1	1	1	1
WT WS - N	2.8	4.3	7.4	2.7	1.3	1.4	0.8	0.7
***ACT8* used as endogenous control**								
WT Col + N	1	1	1	1	1	1	1	1
WT Col - N	1.6	9.04	49.5	1.1	0.2	3.9	0.5	0.4
***Ubq* used as endogenous control**								
WT Col + N	1	1	1	1	1	1	1	1
WT Col - N	1.6	9.04	49.5	1.1	0.2	3.9	0.6	0.4

Hoagland DR. & Arnon DJ. 1950. The water – and agriculture method for growing plants without soil. Calif. Agrip. Exp. Stn. Circular 347.

Table A24. Hoagland solution with nitrogen.

	For 5 l concentrated 10x solution	Equal to 1 l diluted 1x solution	Concentration of nutrition in diluted 1x solution
1 M KH_2PO_4	50 ml	1 ml	1 mM PO_4^{--}
1 M KNO_3	250 ml	5 ml	5 mM NO_3^-
1 M $Ca(NO_3)_2$:4H_2O	250 ml	5 ml	10 mM NO_3^- 5 mM Ca^{++}
$MgSO_4$:7H_2O	100 ml	2 ml	2 mM Mg^{++} 2 mM SO_4^{--}
1 % Fe-EDTA	50 ml	1 ml	
Micronutrition	50 ml	1 ml	

Table A25. Hoagland solution without nitrogen.

	For 5 l concentrated 10x solution	Equal to 1 l diluted 1x solution	Concentration of nutrition in diluted 1x solution
1 M KH_2PO_4	50 ml	1 ml	1 mM PO_4^{--}
1 M KCl	250 ml	5 ml	
1 M $CaCl_2$	250 ml	5 ml	5 mM Ca^{++}
$MgSO_4$:7H_2O	100 ml	2 ml	2 mM Mg^{++} 2 mM SO_4^{--}
1 % Fe-EDTA	50 ml	1 ml	
Micronutrition	50 ml	1 ml	

MS (Murashige and Skoog) medium:

Prepare the following stock solutions:

A.	KNO_3	95 g/l
A.	NH_4N_O3	120 g/l
B.	$MgSO_4$*7H_2O	37 g/l
C.	KH_2PO_4	17 g/l
D.	$CaCl_2$*2H_2O	44 g/

Minor I for 1 l

$ZnSO_4$*7H_2O	0.920 g
H_3BO_3	0.620 g
$MnSO_4$*7H_2O	2.230 g

Minor II for 1 l

$Na_2MoO_4*2H_2O$	0.025 g
$CuSO_4*5H_2O$	0.003 g
$CoCl_2*6_H2O$	0.003 g
KI	0.083 g

Preparation of MS – medium, 1 l:

A	20 ml
B	13 ml
C	10 ml
D	20 ml
E	10 ml
Minor I	10 ml
Minor II	10 ml
Vitamins	5 ml
FE/EDTA	1 ml

For ½ strength MS with nitrogen, 1% sucrose:

MS salts	50 ml
H_2O	50 ml
Agar	0.7 g
Sucrose	0.5

Autoclave. Pour the nutrient solutions into Petri dishes to make agar slant dishes.

For ½ strength MS without nitrogen, 1% sucrose:

MS salts without N (no A & B)	50 ml
1 M KCL	0.5 ml
H_2O	49.5 ml
Agar	0.7 g
Sucrose	0.5

Autoclave. Pour the nutrient solutions into Petri dishes to make agar slant dishes.

Appendix B

Light treatment: 0 = no light, 1 = light

Type: 1) Claree, 2) Susanne, 3) Juanita and 4) Tastery

Treatment: 1) K/N = 0.5 (control), 2) K/N = 7, 3) K/N = 3, 4) K/N = 2

Table B1. The measurements of firmness, sugar concentration (SSC), tiratable acidity (TTA), weight, taste yield/m^2 and fruit soluble yield per m^2 (SSY per m^2) in tomato varieties grown with and without additional light and treated with four different nutrient solutions at Bioforsk Vest, Særheim in 2011.

Week	Light	Type	Treatment	Repetition	Fasthet	SSC (°BRIX)	TTA	Weight (g)	Weight per tomat (g)	Taste	Yield kg/m^2	SSY kg p m^2
33	0	1	4	1	59	5.8	1035.1	89.6	14.9	51.27	0.85	0.05
33	0	1	2	1	61	5.8	884.8	97.1	16.2	43.57	1.12	0.07
33	0	1	3	1	63	5.9	878.7	96.6	16.1	45.23	1.07	0.06
33	0	1	1	1	65	5.5	796.6	101.3	16.9	34.74	1.21	0.07
33	0	1	2	2	62	6.2	864.4	113.7	19.0	50.66	0.97	0.05
33	0	1	3	2	64	5.3	857.3	95.1	15.9	33.67	0.76	0.04
33	0	1	1	2	62	5.4	830.9	78.1	13.0	34.32	1.23	0.07
33	0	1	4	2	64	5.3	761.8	69.4	11.6	30.47	1.11	0.07
33	1	1	4	1	68	6.1	729.7	84	14.0	43.18	1.12	0.07
33	1	1	3	1	62	5.7	750.2	61.2	10.2	36.56	1.06	0.06
33	1	1	2	1	58	6.7	705.0	91.7	15.3	55.08	1.23	0.08
33	1	1	3	2	62	5.9	656.5	87	14.5	37.50	0.79	0.05
33	1	1	1	1	63	5.6	781.0	68	11.3	35.87	1.11	0.06
33	1	1	1	2	63	6.3	816.0	69.4	11.6	50.52	0.92	0.06
33	1	1	2	2	66	6	840.0	74	12.3	45.45	0.85	0.05
33	1	1	4	2	72	5.4	552.6	58.2	9.7	27.85	0.77	0.05
33	0	2	4	1	60	6.4	967.9	169.4	14.1	60.84	0.86	0.05
33	0	2	2	1	64	5.4	758.1	182.4	15.2	31.90	0.82	0.05
33	0	2	3	1	65	6.4	827.0	166.8	13.9	53.18	1.49	0.10
33	0	2	1	1	62	6.4	850.9	184.4	15.4	54.32	1.10	0.08
33	0	2	2	2	62	5.9	895.3	172.8	14.4	46.01	1.34	0.10
33	0	2	3	2	65	6	841.5	184.2	15.4	45.52	1.65	0.11
33	0	2	1	2	63	5.9	800.5	154.9	12.9	41.91	1.76	0.11
33	0	2	4	2	67	6.1	844.1	124.2	10.4	47.63	1.27	0.08
33	1	2	4	1	68	6.3	923.3	130.1	10.8	55.91	0.93	0.05
33	1	2	3	1	69	7	757.7	146.5	12.2	64.21	0.72	0.03
33	1	2	2	1	63	6.9	700.9	147.1	12.3	59.71	0.86	0.04
33	1	2	3	2	65	7.1	667.0	163.8	13.7	63.87	0.70	0.03
33	1	2	1	1	64	6.5	720.5	143.6	12.0	51.09	0.93	0.05
33	1	2	1	2	66	6.5	759.3	144.1	12.0	52.47	0.48	0.02
33	1	2	2	2	67	6.1	713.3	129.9	10.8	42.67	1.06	0.06
33	1	2	4	2	68	7.5	902.3	136.7	11.4	86.07	1.04	0.06
33	0	3	4	1	66	6.2	863.3	65.4	10.9	50.60	1.49	0.08
33	0	3	2	1	70	5.6	742.3	79.7	13.3	34.63	1.30	0.08

33	0	3	3	1	69	4.2	667.5	61.1	10.2	14.99	1.19	0.07
33	0	3	1	1	69	5	672.5	65.8	11.0	24.02	1.22	0.05
33	0	3	2	2	72	4.7	673.0	60.7	10.1	20.33	1.22	0.06
33	0	3	3	2	71	5.4	551.5	62.1	10.4	27.84	1.49	0.08
33	0	3	1	2	67	5.2	770.7	55.1	9.2	29.25	1.42	0.08
33	0	3	4	2	74	5.7	714.9	54.5	9.1	35.49	1.34	0.08
33	1	3	4	1	70	5.3	647.3	46.5	7.8	27.63	1.16	0.06
33	1	3	3	1	73	5.4	671.4	51.2	8.5	29.61	0.91	0.05
33	1	3	2	1	67	5.9	621.1	54.1	9.0	36.84	1.06	0.06
33	1	3	3	2	69	5.1	530.9	61.6	10.3	23.43	1.46	0.09
33	1	3	1	1	66	6	542.0	49.5	8.3	38.06	1.46	0.09
33	1	3	1	2	70	5.6	646.0	46.9	7.8	32.19	1.35	0.08
33	1	3	2	2	73	4.4	767.7	43.6	7.3	18.96	1.16	0.07
33	1	3	4	2	71	4.8	664.8	52.3	8.7	21.36	1.02	0.06
33	0	4	4	1	81	6.2	642.6	143.7	24.0	42.86	0.89	0.05
33	0	4	2	1	81	5.1	560.4	129.7	21.6	23.63	1.08	0.06
33	0	4	3	1	81	5.6	569.1	148.5	24.8	31.11	0.90	0.05
33	0	4	1	1	82	5.7	634.4	136.4	22.7	33.61	1.03	0.06
33	0	4	2	2	76	5.8	567.1	138.2	23.0	34.47	0.83	0.04
33	0	4	3	2	81	5.1	578.0	140.9	23.5	23.80	0.80	0.04
33	0	4	1	2	77	5.2	554.6	105	17.5	24.95	0.99	0.05
33	0	4	4	2	77	4.9	625.7	79.4	13.2	21.89	1.18	0.06
33	1	4	4	1	77	6.4	600.7	95.6	15.9	46.36	1.64	0.08
33	1	4	3	1	74	6.1	543.8	107.6	17.9	40.00	1.23	0.07
33	1	4	2	1	79	6.2	522.5	120	20.0	42.16	1.23	0.07
33	1	4	3	2	78	6.2	553.8	142.5	23.8	41.99	1.02	0.06
33	1	4	1	1	81	5.9	485.1	111.7	18.6	36.65	0.90	0.05
33	1	4	1	2	80	6	744.8	93.7	15.6	41.77	0.54	0.03
33	1	4	2	2	75	5.5	460.5	95.9	16.0	29.81	0.67	0.04
33	1	4	4	2	77	5.6	552.0	105.7	17.6	31.00	0.52	0.03
34	0	1	4	1	64	5.9	807.1	88.6	14.8	42.17	0.68	0.04
34	0	1	2	1	66	5.4	704.9	73.8	12.3	30.41	0.60	0.03
34	0	1	3	1	64	5.8	844.1	92.3	15.4	41.80	1.23	0.07
34	0	1	1	1	81	5.1	614.4	85.9	14.3	24.28	1.15	0.07
34	0	1	2	2	64	5.9	519.3	90.5	15.1	36.26	1.57	0.10
34	0	1	3	2	64	5.3	759.8	63.1	10.5	30.41	1.42	0.10
34	0	1	1	2	59	5.1	718.0	73.8	12.3	26.39	1.19	0.08
34	0	1	4	2	60	5.8	903.5	65.2	10.9	44.43	0.82	0.06
34	1	1	4	1	67	5.2	835.3	67.4	11.2	31.32	0.95	0.05
34	1	1	3	1	59	5.3	864.1	56.8	9.5	33.92	0.65	0.03
34	1	1	2	1	66	5.4	897.7	62.7	10.5	36.90	0.60	0.03
34	1	1	3	2	64	4.9	858.0	64.4	10.7	27.66	0.53	0.02
34	1	1	1	1	66	5.4	814.1	71.5	11.9	33.72	0.86	0.04
34	1	1	1	2	65	5.7	872.5	82.9	13.8	41.16	0.34	0.01
34	1	1	2	2	64	5.7	946.7	71.4	11.9	44.56	0.87	0.04
34	1	1	4	2	65	5.7	886.8	72	12.0	41.78	1.00	0.05
34	0	2	4	1	69	6.6	857.7	168.7	14.1	59.24	1.02	0.05
34	0	2	2	1	64	5.5	978.0	149.9	12.5	42.25	1.04	0.05
34	0	2	3	1	73	6.1	652.6	133.8	11.2	41.12	0.69	0.04
34	0	2	1	1	67	6.1	555.0	162.3	13.5	39.99	0.64	0.03
34	0	2	2	2	66	5.5	1205.7	131.8	11.0	54.79	0.65	0.03

34	0	2	3	2	66	5.4	914.0	135.6	11.3	37.57	1.09	0.06
34	0	2	1	2	65	5.7	870.4	149.1	12.4	41.07	0.86	0.05
34	0	2	4	2	63	5.3	869.5	107.4	9.0	34.12	0.87	0.05
34	1	2	4	1	69	5.8	658.5	114.4	9.5	35.79	1.24	0.06
34	1	2	3	1	69	5.8	767.2	66.1	5.5	38.87	0.34	0.02
34	1	2	2	1	66	6.4	1122.0	123.9	10.3	71.46	1.24	0.06
34	1	2	3	2	66	6.4	736.6	149.6	12.5	49.49	1.56	0.08
34	1	2	1	1	65	6.8	900.5	139.2	11.6	66.54	1.17	0.07
34	1	2	1	2	69	6.4	758.4	169.6	13.0	50.27	1.27	0.07
34	1	2	2	2	74	6.8	950.7	130.9	10.9	69.73	0.91	0.05
34	1	2	4	2	68	7.2	939.6	141.1	11.8	79.90	0.87	0.05
34	0	3	4	1	74	5.3	743.7	54	9.0	29.94	1.11	0.07
34	0	3	2	1	71	5.3	579.4	68	11.3	26.60	1.02	0.06
34	0	3	3	1	69	4.6	666.8	53.4	8.9	19.08	1.20	0.07
34	0	3	1	1	73	5.2	651.0	51.1	8.5	26.28	1.05	0.06
34	0	3	2	2	76	4.3	650.0	58.9	9.8	15.67	0.88	0.05
34	0	3	3	2	74	4.4	710.1	60.1	10.0	17.74	0.98	0.05
34	0	3	1	2	70	4.3	706.9	37.1	6.2	16.65	1.60	0.09
34	0	3	4	2	71	5.2	747.5	59.8	10.0	28.58	1.46	0.09
34	1	3	4	1	70	5.3	692.1	31.6	5.3	28.60	2.22	0.13
34	1	3	3	1	76	4.9	635.1	51.5	8.6	22.04	2.20	0.15
34	1	3	2	1	71	4.7	678.5	45.4	7.6	20.44	1.53	0.10
34	1	3	3	2	70	5.3	597.1	49.7	8.3	26.81	1.51	0.09
34	1	3	1	1	71	4.8	755.7	45.2	7.5	23.38	1.00	0.06
34	1	3	1	2	74	5.1	683.4	46.4	7.7	25.58	0.67	0.04
34	1	3	2	2	73	5.1	670.1	36.2	6.0	25.29	0.98	0.06
34	1	3	4	2	73	5.4	630.6	41.5	6.9	28.80	0.84	0.05
34	0	4	4	1	85	6.3	567.9	130.3	21.7	44.06	0.93	0.06
34	0	4	2	1	78	5.1	536.5	111.7	18.6	23.46	0.74	0.04
34	0	4	3	1	69	5.4	686.5	124.1	20.7	29.95	1.83	0.12
34	0	4	1	1	80	5.8	787.0	128.5	21.4	39.57	1.72	0.11
34	0	4	2	2	76	5.5	595.3	111.1	18.5	29.79	1.60	0.11
34	0	4	3	2	77	5	489.5	95.4	15.9	22.03	1.76	0.13
34	0	4	1	2	74	5.2	486.3	93.4	15.6	24.83	1.37	0.09
34	0	4	4	2	73	4.9	612.8	65.9	11.0	21.69	1.35	0.09
34	1	4	4	1	81	5.5	615.2	86.5	14.4	30.07	1.02	0.06
34	1	4	3	1	79	5	759.3	90.2	15.0	26.10	0.68	0.03
34	1	4	2	1	80	5	467.1	122.7	17.5	22.13	0.71	0.04
34	1	4	3	2	79	5.6	740.9	119.8	20.0	34.58	0.62	0.03
34	1	4	1	1	81	5.4	561.5	101.9	14.6	27.91	1.27	0.06
34	1	4	1	2	81	5.6	588.1	116.5	19.4	31.29	0.50	0.03
34	1	4	2	2	79	5.5	528.0	81.1	13.5	29.32	1.15	0.06
34	1	4	4	2	80	5.5	514.5	93.9	15.7	29.33	1.23	0.08
35	0	1	4	1	67	6.9	856.2	73.4	12.2	66.50	1.66	0.10
35	0	1	2	1	63	6.2	832.0	72.8	12.1	49.14	1.40	0.09
35	0	1	3	1	64	6	701.0	76.3	12.7	40.42	1.06	0.05
35	0	1	1	1	61	6.2	911.6	76.2	12.7	53.05	0.78	0.04
35	0	1	2	2	47	5.8	731.7	73.1	12.2	37.71	0.90	0.05
35	0	1	3	2	67	5.3	635.6	59.6	9.9	27.41	1.28	0.07
35	0	1	1	2	63	5.5	776.3	67.3	11.2	34.06	1.60	0.09
35	0	1	4	2	62	5.1	838.7	62.1	10.4	29.92	1.41	0.08

35	1	1	4	1	69	5.9	698.0	57.8	9.6	38.51	1.09	0.06
35	1	1	3	1	60	5.9	883.5	80.5	13.4	45.45	0.72	0.04
35	1	1	2	1	61	6.2	640.9	75.5	12.6	42.83	1.08	0.06
35	1	1	3	2	61	6	703.2	79.9	13.3	40.48	1.94	0.12
35	1	1	1	1	60	6.7	757.0	75.6	12.6	56.91	1.82	0.12
35	1	1	1	2	67	6.3	672.1	74.5	12.4	45.50	1.51	0.09
35	1	1	2	2	65	5.7	900.9	71.3	11.9	42.41	1.39	0.08
35	1	1	4	2	65	6.1	751.7	71.3	11.9	43.92	1.17	0.07
35	0	2	4	1	70	6.8	910.2	138.8	11.6	67.14	0.54	0.03
35	0	2	2	1	65	6.1	820.8	133.2	11.1	46.61	0.48	0.03
35	0	2	3	1	72	6.1	753.7	138.6	11.6	43.98	0.60	0.04
35	0	2	1	1	67	6	873.0	130.7	10.9	46.94	0.53	0.03
35	0	2	2	2	70	5.6	870.6	112	9.3	39.27	0.40	0.02
35	0	2	3	2	68	6.2	725.1	137.3	11.4	44.99	0.46	0.03
35	0	2	1	2	65	5.9	696.5	131.9	11.0	38.47	1.17	0.06
35	0	2	4	2	69	6.2	815.1	117.7	9.8	48.39	0.84	0.05
35	1	2	4	1	66	6.4	730.4	122.1	10.2	49.27	0.94	0.06
35	1	2	3	1	66	6.7	731.3	140.7	11.7	55.95	0.99	0.07
35	1	2	2	1	67	6.5	723.5	133.7	11.1	51.19	0.82	0.05
35	1	2	3	2	64	7.1	677.5	166.1	13.8	64.11	0.77	0.05
35	1	2	1	1	66	7.3	705.2	141.3	11.8	70.20	0.43	0.03
35	1	2	1	2	66	6.9	633.5	166.8	13.9	58.21	0.31	0.02
35	1	2	2	2	72	6.5	832.5	104.4	8.7	55.67	0.44	0.03
35	1	2	4	2	67	7.2	782.0	142	11.8	70.49	0.38	0.02
35	0	3	4	1	74	5.7	827.2	39.7	6.6	39.30	0.37	0.02
35	0	3	2	1	72	5.6	758.4	63	10.5	35.13	0.33	0.02
35	0	3	3	1	72	4.9	625.1	40.4	6.7	21.88	0.69	0.05
35	0	3	1	1	73	5.2	718.6	45.2	7.5	27.81	0.64	0.04
35	0	3	2	2	75	4.8	687.7	39.5	6.6	21.81	0.60	0.04
35	0	3	3	2	72	4.9	718.8	55.6	9.3	23.75	0.75	0.05
35	0	3	1	2	67	5.3	844.0	41.6	6.9	33.18	0.55	0.03
35	0	3	4	2	75	4.8	886.8	57.6	9.6	27.19	0.55	0.04
35	1	3	4	1	69	6.6	672.0	35.8	6.0	51.91	0.48	0.03
35	1	3	3	1	69	5.2	613.4	54.1	9.0	25.63	0.40	0.02
35	1	3	2	1	71	6.4	635.6	54.5	9.1	46.82	0.40	0.02
35	1	3	3	2	70	5.9	552.9	45.9	7.7	36.20	0.26	0.02
35	1	3	1	1	70	6.1	600.8	48.5	8.1	40.28	0.44	0.02
35	1	3	1	2	68	4.9	527.2	44	7.3	20.81	0.18	0.01
35	1	3	2	2	72	5.5	796.6	41.4	6.9	34.74	0.59	0.04
35	1	3	4	2	70	5.5	702.7	42.4	7.1	31.90	0.55	0.03
35	0	4	4	1	82	6.3	659.3	97.3	16.2	45.21	0.53	0.02
35	0	4	2	1	79	5.7	547.6	82.9	13.8	32.65	0.49	0.03
35	0	4	3	1	78	5.5	624.0	96.7	16.1	30.21	0.64	0.03
35	0	4	1	1	79	5.9	668.6	127.7	21.3	37.77	0.46	0.02
35	0	4	2	2	81	5.4	697.8	110.6	18.4	30.23	0.49	0.03
35	0	4	3	2	78	5.4	730.8	85.8	14.3	31.10	0.52	0.03
35	0	4	1	2	75	5.3	585.4	66.7	11.1	26.67	0.56	0.03
35	0	4	4	2	77	4.8	726.6	57.6	9.6	22.67	0.53	0.03
35	1	4	4	1	81	6	574.5	93.5	15.6	38.14	0.50	0.03
35	1	4	3	1	78	5.7	586.5	88	14.7	32.92	0.35	0.02
35	1	4	2	1	79	6.3	681.5	118.1	19.7	45.74	0.88	0.05

35	1	4	3	2	77	6.7	549.5	124	20.7	53.14	1.27	0.08
35	1	4	1	1	80	6.3	745.0	95.3	15.9	47.69	1.27	0.07
35	1	4	1	2	82	5.8	424.8	101.6	16.9	36.35	1.14	0.07
35	1	4	2	2	78	6.2	660.0	76.5	12.8	43.22	1.03	0.06
35	1	4	4	2	77	6.3	619.8	95.6	15.9	44.49	0.59	0.04
36	0	1	4	1	65	6.2	945.0	62.8	10.5	54.88	0.66	0.04
36	0	1	2	1	63	6	908.9	76.2	12.7	48.68	0.67	0.04
36	0	1	3	1	62	6	863.0	52.3	8.7	46.48	0.67	0.04
36	0	1	1	1	61	6	796.7	73	12.2	43.66	0.78	0.05
36	0	1	2	2	63	6	925.3	61.9	10.3	49.52	0.48	0.03
36	0	1	3	2	61	5.2	792.6	59.2	9.9	29.92	0.54	0.03
36	0	1	1	2	64	5.7	915.3	51.2	8.5	43.07	1.16	0.06
36	0	1	4	2	66	5.6	930.3	47.5	7.9	41.88	1.09	0.07
36	1	1	4	1	65	5.4	932.3	51.5	8.6	38.35	1.55	0.09
36	1	1	3	1	64	5.3	672.5	54.4	9.1	28.15	1.40	0.09
36	1	1	2	1	65	5.8	836.1	70.6	11.8	41.47	1.26	0.08
36	1	1	3	2	67	6	855.9	86.8	14.5	46.16	1.13	0.07
36	1	1	1	1	67	6.6	818.7	62.3	10.4	57.28	0.49	0.03
36	1	1	1	2	65	5.8	843.6	78.4	13.1	41.78	0.45	0.03
36	1	1	2	2	67	6.2	921.3	63	10.5	53.58	0.56	0.03
36	1	1	4	2	67	5.6	644.2	73.6	12.3	32.16	0.55	0.03
36	0	2	4	1	65	6.5	859.1	127.4	10.6	56.99	0.51	0.03
36	0	2	2	1	66	6.8	885.6	112.8	9.4	65.65	0.51	0.03
36	0	2	3	1	70	6.3	969.3	101.7	8.5	58.57	1.26	0.08
36	0	2	1	1	68	6	800.5	126.8	10.6	43.81	1.35	0.10
36	0	2	2	2	67	6.5	866.5	99.2	8.3	57.38	1.31	0.09
36	0	2	3	2	64	6.2	841.5	123.9	10.3	49.57	1.40	0.09
36	0	2	1	2	65	5.2	728.3	93	7.8	28.06	1.24	0.09
36	0	2	4	2	71	6.4	914.8	55	4.6	57.71	0.67	0.04
36	1	2	4	1	63	6.5	839.4	106.1	8.8	56.00	0.63	0.03
36	1	2	3	1	70	6.7	763.5	92.5	7.7	57.16	0.47	0.02
36	1	2	2	1	68	6.9	798.4	128.5	10.7	63.51	0.45	0.02
36	1	2	3	2	73	6.8	819.9	150	12.5	62.09	0.48	0.03
36	1	2	1	1	68	6.5	591.5	106.1	8.8	48.45	0.61	0.03
36	1	2	1	2	67	6.3	826.0	126.6	10.6	50.97	0.34	0.02
36	1	2	2	2	71	6.5	932.0	109	9.1	61.05	0.99	0.06
36	1	2	4	2	66	6.5	741.3	123	10.3	51.80	0.89	0.05
36	0	3	4	1	71	5	762.9	29.2	4.9	26.20	0.94	0.06
36	0	3	2	1	71	6	726.6	58.4	9.7	41.18	0.72	0.04
36	0	3	3	1	74	5.2	792.5	43.4	7.2	29.91	0.50	0.03
36	0	3	1	1	75	5.6	724.2	46.2	7.7	34.09	0.51	0.02
36	0	3	2	2	77	6.1	942.5	32.2	5.4	52.55	0.54	0.03
36	0	3	3	2	70	4.7	706.9	50.5	8.4	21.02	0.63	0.04
36	0	3	1	2	72	4.8	853.6	36.7	6.1	26.14	0.91	0.05
36	0	3	4	2	73	5	836.9	42.8	7.1	28.40	0.65	0.04
36	1	3	4	1	71	4.5	732.8	42.9	7.2	19.29	0.53	0.03
36	1	3	3	1	72	6.1	639.7	49.3	8.2	40.86	0.28	0.01
36	1	3	2	1	73	5.4	702.5	49.4	8.2	30.35	1.05	0.06
36	1	3	3	2	74	4.6	413.2	37.3	6.2	17.53	1.15	0.08
36	1	3	1	1	73	5.5	647.6	42.8	7.1	30.64	1.60	0.09
36	1	3	1	2	72	4.8	504.9	28.3	4.7	19.50	1.12	0.07

36	1	3	2	2	74	4.9	805.7	35.8	6.0	26.05	0.95	0.05
36	1	3	4	2	73	5.3	722.7	40	6.7	29.37	0.67	0.04
36	0	4	4	1	84	6	660.7	103.8	17.3	39.41	0.28	0.02
36	0	4	2	1	82	5.5	728.3	74.8	12.5	32.59	0.10	0.01
36	0	4	3	1	83	6	663.6	87.3	14.6	39.48	0.29	0.02
36	0	4	1	1	83	5.7	647.1	116.3	19.4	33.85	0.18	0.01
36	0	4	2	2	80	5.7	720.1	95.4	15.9	35.64	0.11	0.01
36	0	4	3	2	79	5.9	941.0	79	13.2	48.26	0.14	0.01
36	0	4	1	2	77	4.9	884.6	62.7	10.5	28.54	0.75	0.04
36	0	4	4	2	70	5.3	829.0	55	9.2	32.65	0.62	0.04
36	1	4	4	1	73	5.4	.	94	15.7	.	0.73	0.04
36	1	4	3	1	81	5.5	603.5	89.3	14.9	29.90	0.91	0.06
36	1	4	2	1	83	6.6	562.7	112.9	18.8	50.68	0.61	0.03
36	1	4	3	2	82	5.9	568.3	130.9	21.8	36.26	1.14	0.07
36	1	4	1	1	84	6.2	559.1	109.7	18.3	41.99	0.13	0.01
36	1	4	1	2	83	6	663.9	102.4	17.1	39.48	0.15	0.01
36	1	4	2	2	82	6.1	569.6	75	12.5	40.03	0.16	0.01
36	1	4	4	2	78	5.6	825.1	98.1	16.4	37.46	0.17	0.01
37	0	1	4	1	65	6.3	1030.8	61.2	10.2	62.45	0.10	0.01
37	0	1	2	1	64	6.2	836.6	66.4	11.1	49.35	0.17	0.01
37	0	1	3	1	66	5.9	854.9	64.1	10.7	44.16	0.82	0.06
37	0	1	1	1	64	6.3	883.8	71.9	12.0	53.78	1.03	0.07
37	0	1	2	2	68	5.8	783.0	68.2	11.4	39.43	0.79	0.05
37	0	1	3	2	64	5.6	816.0	48.4	8.1	37.12	0.77	0.05
37	0	1	1	2	62	6	871.8	53.4	8.9	46.89	0.97	0.06
37	0	1	4	2	62	5.1	745.2	41	6.8	27.09	0.46	0.03
37	1	1	4	1	66	6.5	705.4	65.2	10.9	50.62	0.40	0.02
37	1	1	3	1	65	5.6	702.6	39.1	6.5	33.50	0.20	0.01
37	1	1	2	1	67	6.3	650.6	103.8	17.3	45.03	0.10	0.01
37	1	1	3	2	66	5.5	597.1	71.8	12.0	29.81	0.10	0.00
37	1	1	1	1	65	6.4	664.6	71	11.8	47.40	0.13	0.01
37	1	1	1	2	66	6	665.1	72.5	12.1	39.51	0.06	0.00
37	1	1	2	2	69	5.8	746.9	51	8.5	38.19	0.65	0.04
37	1	1	4	2	63	6	644.2	69.6	11.6	39.07	0.52	0.03
37	0	2	4	1	69	6.9	960.3	141	11.8	73.03	0.57	0.03
37	0	2	2	1	66	6.1	814.3	100.3	8.4	46.33	0.41	0.02
37	0	2	3	1	72	5.9	761.6	80.5	8.1	40.48	0.28	0.01
37	0	2	1	1	68	6.1	976.5	114	9.5	54.45	0.36	0.02
37	0	2	2	2	66	6.3	1019.2	104	9.5	61.69	0.34	0.02
37	0	2	3	2	63	5.9	844.8	94.9	7.9	43.72	0.35	0.02
37	0	2	1	2	64	5.7	758.2	39.7	9.9	36.81	0.46	0.03
37	0	2	4	2	68	6.2	809.6	85	7.1	48.16	0.44	0.02
37	1	2	4	1	72	6.6	719.7	123.4	10.3	53.27	0.30	0.02
37	1	2	3	1	70	6.2	587.9	105.9	8.8	42.12	0.26	0.01
37	1	2	2	1	72	7.2	714.7	148	12.3	67.80	0.66	0.04
37	1	2	3	2	68	6.5	655.1	137.9	11.5	49.33	0.70	0.04
37	1	2	1	1	67	6.6	570.0	145.1	12.1	50.66	0.85	0.05
37	1	2	1	2	67	7	1048.0	106.6	8.9	82.44	0.54	0.03
37	1	2	2	2	73	6.7	726.9	100.4	10.0	55.79	0.73	0.04
37	1	2	4	2	66	6.3	722.4	149	12.4	46.93	0.71	0.04
37	0	3	4	1	73	5.8	736.1	41.4	6.9	37.85	0.61	0.04

37	0	3	2	1	74	5.3	691.7	49.1	8.2	28.59	0.33	0.02
37	0	3	3	1	74	5.1	767.3	41	6.8	27.71	0.27	0.02
37	0	3	1	1	71	5.2	722.4	43.2	7.2	27.90	0.31	0.02
37	0	3	2	2	75	5.2	816.5	34.4	5.7	30.68	0.28	0.01
37	0	3	3	2	71	5.2	671.6	49.3	8.2	26.70	0.19	0.01
37	0	3	1	2	69	5.4	873.0	40.4	6.7	35.91	0.87	0.06
37	0	3	4	2	76	5.3	668.0	39.9	6.7	28.05	0.93	0.05
37	1	3	4	1	77	5	602.5	34.4	5.7	22.80	0.86	0.05
37	1	3	3	1	73	5.8	615.2	46.7	7.8	34.99	1.18	0.08
37	1	3	2	1	74	5.5	703.9	38	6.3	31.93	0.80	0.05
37	1	3	3	2	73	6	517.2	42.3	7.1	38.18	1.01	0.06
37	1	3	1	1	71	5.4	570.0	33.3	5.6	27.98	0.47	0.03
37	1	3	1	2	70	5.2	684.2	29.1	4.9	26.98	0.26	0.02
37	1	3	2	2	74	4.7	694.5	30.9	5.2	20.76	0.23	0.01
37	1	3	4	2	73	5.7	548.0	41.8	7.0	32.65	0.21	0.01
37	0	4	4	1	86	6	695.5	94.8	15.8	40.27	0.28	0.02
37	0	4	2	1	80	6	691.7	67.1	11.2	40.17	0.33	0.02
37	0	4	3	1	82	5.8	827.5	78.2	13.0	41.12	0.76	0.05
37	0	4	1	1	85	5.4	779.6	105.8	17.6	32.57	1.09	0.08
37	0	4	2	2	83	5.4	628.9	104	17.3	28.77	0.76	0.05
37	0	4	3	2	82	5.8	635.3	78.4	13.1	35.33	0.79	0.05
37	0	4	1	2	77	5.1	757.4	64.9	10.8	27.43	0.86	0.06
37	0	4	4	2	78	5.4	806.2	57.7	9.6	33.45	0.64	0.04
37	1	4	4	1	82	6	637.2	93.2	15.5	38.94	0.29	0.01
37	1	4	3	1	85	5.6	424.8	68.8	11.5	32.46	0.28	0.01
37	1	4	2	1	83	6.7	554.4	86.7	14.5	53.09	0.31	0.01
37	1	4	3	2	85	5.8	417.2	71.2	11.9	36.67	0.29	0.02
37	1	4	1	1	84	6	385.6	89.4	14.9	42.95	0.44	0.02
37	1	4	1	2	82	5.3	447.5	77.3	12.9	26.75	0.27	0.01
37	1	4	2	2	81	5.7	625.2	61.3	10.2	33.45	0.62	0.04
37	1	4	4	2	81	5.9	453.3	89.4	14.9	37.38	0.60	0.03
38	0	1	4	1	63	6.8	922.3	44.6	7.4	67.89	0.63	0.04
38	0	1	2	1	66	6.1	735.1	51.6	8.6	43.35	0.64	0.03
38	0	1	3	1	65	5.5	987.1	48.2	8.0	42.69	0.30	0.01
38	0	1	1	1	68	6.1	726.3	61	10.2	43.07	0.37	0.02
38	0	1	2	2	64	5.2	1093.1	49.7	8.3	42.20	0.34	0.02
38	0	1	3	2	72	5.8	803.9	52.7	8.8	40.20	0.24	0.01
38	0	1	1	2	69	5.6	803.9	44.7	7.5	36.68	0.70	0.04
38	0	1	4	2	68	5.5	921.1	46.1	7.7	39.64	0.40	0.02
38	1	1	4	1	63	6.2	847.2	57.6	9.6	49.84	0.37	0.02
38	1	1	3	1	63	5.9	767.8	59.1	9.9	40.70	0.30	0.02
38	1	1	2	1	63	6.3	777.0	60.8	10.1	48.88	0.71	0.04
38	1	1	3	2	66	5.1	477.1	43.6	7.3	23.45	0.84	0.05
38	1	1	1	1	68	6.3	804.8	58.5	9.8	50.03	0.86	0.05
38	1	1	1	2	66	5.2	852.8	66.7	11.1	31.93	0.88	0.05
38	1	1	2	2	68	5.9	892.6	63.9	10.7	45.88	0.80	0.04
38	1	1	4	2	62	5.6	782.3	56.2	9.4	35.92	0.64	0.03
38	0	2	4	1	73	6.8	950.0	81.3	6.8	69.68	0.65	0.04
38	0	2	2	1	65	6.7	857.0	96.6	8.8	61.58	0.31	0.02
38	0	2	3	1	71	6	820.1	96	8.0	44.61	0.91	0.06
38	0	2	1	1	71	6	1032.6	109.5	9.1	55.52	0.60	0.03

38	0	2	2	2	69	5.7	862.0	117.8	9.8	40.71	0.46	0.03
38	0	2	3	2	67	5.7	841.7	71.5	6.0	39.88	0.58	0.03
38	0	2	1	2	70	5.6	864.4	91.6	7.6	39.01	0.91	0.06
38	0	2	4	2	70	5.6	883.3	55	6.9	39.80	0.87	0.05
38	1	2	4	1	69	6.1	834.7	69	8.6	47.21	0.95	0.06
38	1	2	3	1	68	6.9	662.2	95.1	7.9	58.72	1.19	0.07
38	1	2	2	1	71	6.9	946.4	111	9.3	72.08	1.09	0.07
38	1	2	3	2	67	6	691.9	67.6	11.3	40.17	1.11	0.08
38	1	2	1	1	68	7.1	759.4	127.1	10.6	66.85	0.45	0.03
38	1	2	1	2	67	6.4	694.0	110	9.2	48.15	0.47	0.03
38	1	2	2	2	71	6.2	905.9	114.1	9.5	52.75	0.64	0.04
38	1	2	4	2	65	6.8	747.7	90.5	11.3	58.90	0.48	0.03
38	0	3	4	1	68	5.2	841.2	30.9	5.2	31.52	0.40	0.02
38	0	3	2	1	71	5.8	784.8	43.2	7.2	39.49	0.40	0.02
38	0	3	3	1	69	5.5	756.6	34.3	5.7	33.43	1.08	0.07
38	0	3	1	1	70	5.4	677.7	31.8	5.3	29.75	1.17	0.08
38	0	3	2	2	77	5.1	870.8	33.7	5.6	31.03	1.18	0.08
38	0	3	3	2	71	4.9	804.0	44.2	7.4	26.00	1.06	0.07
38	0	3	1	2	72	5.1	739.6	42.4	7.1	26.94	1.08	0.07
38	0	3	4	2	75	5.1	768.3	41.9	7.0	27.74	0.65	0.04
38	1	3	4	1	73	5.3	684.0	38.5	6.4	28.41	0.70	0.03
38	1	3	3	1	73	6.6	648.8	42.1	7.0	51.41	0.31	0.01
38	1	3	2	1	75	4.9	766.7	39.2	6.5	24.96	0.37	0.02
38	1	3	3	2	74	6.1	647.8	38.1	6.4	41.02	0.44	0.02
38	1	3	1	1	72	5.4	697.5	32.4	5.4	30.22	0.40	0.02
38	1	3	1	2	75	5	700.1	30	5.0	24.61	0.36	0.02
38	1	3	2	2	72	5.2	802.5	31.9	5.3	30.23	0.85	0.05
38	1	3	4	2	73	5.4	713.9	37.8	6.3	30.64	0.48	0.03
38	0	4	4	1	84	6.1	768.0	89.5	14.9	44.50	0.66	0.04
38	0	4	2	1	82	5.7	704.5	67.4	11.2	35.21	0.53	0.03
38	0	4	3	1	83	6.2	777.6	80.4	13.4	46.86	0.33	0.02
38	0	4	1	1	78	6.4	779.1	93.1	15.5	51.08	0.45	0.02
38	0	4	2	2	82	5.5	733.3	87.8	14.6	32.74	0.70	0.04
38	0	4	3	2	84	5.8	758.1	85	14.2	38.56	0.72	0.04
38	0	4	1	2	76	5.1	851.9	56.5	9.4	30.37	1.05	0.07
38	0	4	4	2	77	5.5	853.0	57	9.5	36.82	0.90	0.04
38	1	4	4	1	81	5.9	612.7	90.6	15.1	36.72	0.71	0.04
38	1	4	3	1	84	5.6	547.2	83.4	13.9	30.98	0.56	0.03
38	1	4	2	1	85	6.2	426.1	85.6	14.3	45.07	1.06	0.06
38	1	4	3	2	85	5.9	456.3	108.3	18.1	37.30	0.96	0.06
38	1	4	1	1	85	5.7	599.3	87.1	14.5	33.07	0.95	0.06
38	1	4	1	2	82	5.2	548.9	100.9	16.8	24.91	1.04	0.06
38	1	4	2	2	80	5.2	622.9	62.7	10.5	25.78	0.66	0.00
38	1	4	4	2	82	5.8	556.7	94.2	15.7	34.42	0.74	0.04
39	0	1	4	1	65	6.8	910.2	60.3	10.1	67.13	0.52	0.03
39	0	1	2	1	66	6.4	686.2	62.5	10.4	47.93	0.31	0.02
39	0	1	3	1	65	6.3	746.1	55.7	9.3	47.73	0.61	0.04
39	0	1	1	1	63	6.5	740.9	67	11.2	51.79	0.40	0.02
39	0	1	2	2	67	5.6	955.8	67.3	11.2	43.08	0.33	0.02
39	0	1	3	2	68	4.6	777.7	74.2	12.4	21.48	0.31	0.02
39	0	1	1	2	68	6.2	841.1	53.3	8.9	49.55	1.05	0.06

39	0	1	4	2	70	5.4	1014.3	53.4	8.9	42.12	0.80	0.04
39	1	1	4	1	65	6	642.3	48.2	8.0	39.03	1.07	0.06
39	1	1	3	1	63	6.4	518.8	67.2	11.2	46.53	1.34	0.09
39	1	1	2	1	67	5.3	646.4	52	8.7	27.61	0.88	0.05
39	1	1	3	2	63	5.6	693.8	65.1	10.9	33.27	1.04	0.06
39	1	1	1	1	64	6.4	686.1	59.3	9.9	47.93	0.34	0.02
39	1	1	1	2	.	6.1	620.9	64.4	10.7	40.55	0.35	0.02
39	1	1	2	2	63	5.8	783.1	68.2	11.4	39.43	0.41	0.03
39	1	1	4	2	64	6.2	877.2	48.5	8.1	51.28	0.31	0.02
39	0	2	4	1	68	7	954.4	124.5	10.4	75.35	0.32	0.02
39	0	2	2	1	69	6.9	807.4	96.1	8.0	63.94	0.28	0.02
39	0	2	3	1	75	6.2	790.9	67.5	5.6	47.38	0.90	0.05
39	0	2	1	1	68	5.9	979.2	103.9	8.7	50.28	0.80	0.05
39	0	2	2	2	67	6	996.7	93.5	7.8	53.41	1.14	0.07
39	0	2	3	2	69	5.6	836.7	89.4	7.5	37.91	0.83	0.05
39	0	2	1	2	70	6.1	835.6	82.3	6.9	47.25	0.90	0.06
39	0	2	4	2	69	6.1	888.0	78.3	7.8	49.71	0.66	0.04
39	1	2	4	1	68	6.1	790.5	94.5	7.9	45.36	0.36	0.02
39	1	2	3	1	66	6.7	571.3	118.1	9.8	53.00	0.29	0.01
39	1	2	2	1	72	7	782.6	105.5	8.8	65.29	0.40	0.02
39	1	2	3	2	71	6	618.3	118.1	9.8	38.62	0.36	0.02
39	1	2	1	1	66	6.9	741.7	129.2	10.8	61.09	0.32	0.02
39	1	2	1	2	67	7.1	698.0	102.6	8.6	64.66	0.38	0.02
39	1	2	2	2	72	6.5	894.7	85	7.1	58.90	0.65	0.04
39	1	2	4	2	67	6.1	646.1	89	7.4	40.99	0.54	0.03
39	0	3	4	1	75	5.9	734.5	39.5	6.6	39.59	0.53	0.02
39	0	3	2	1	75	4.9	660.1	41.9	7.0	22.49	0.45	0.02
39	0	3	3	1	72	4.5	691.2	28.7	4.8	18.44	0.46	0.02
39	0	3	1	1	75	4.9	809.6	28.2	4.7	26.17	0.44	0.02
39	0	3	2	2	71	5.2	873.3	30.1	5.0	32.67	0.40	0.02
39	0	3	3	2	74	5.4	772.4	40	6.7	32.34	0.24	0.01
39	0	3	1	2	74	4.7	799.9	34.8	5.8	23.28	0.77	0.05
39	0	3	4	2	71	6.1	730.1	31.9	5.3	43.19	0.36	0.02
39	1	3	4	1	73	4	551.9	35.5	5.9	11.78	0.46	0.03
39	1	3	3	1	73	6.6	525.1	49.4	8.2	51.06	0.11	0.01
39	1	3	2	1	72	5	569.2	32.7	5.5	22.40	0.91	0.05
39	1	3	3	2	75	6.1	434.1	42.7	7.1	42.36	0.88	0.05
39	1	3	1	1	73	5.1	771.8	34.9	5.8	27.84	0.71	0.04
39	1	3	1	2	76	4.8	645.9	25.3	4.2	21.01	0.73	0.04
39	1	3	2	2	72	5	830.8	28.3	4.7	28.21	0.69	0.04
39	1	3	4	2	73	4.8	705.3	28.7	4.8	22.19	0.75	0.04
39	0	4	4	1	88	6.5	675.1	85	14.2	49.79	0.05	0.00
39	0	4	2	1	82	5.9	785.8	56.2	9.4	41.35	0.04	0.00
39	0	4	3	1	81	5.9	747.7	72.8	12.1	40.01	0.05	0.00
39	0	4	1	1	84	6.2	644.8	100	16.7	42.90	0.04	0.00
39	0	4	2	2	81	6	615.6	82	13.7	38.58	0.04	0.00
39	0	4	3	2	86	5.8	681.4	85.9	14.3	36.32	0.03	0.00
39	0	4	1	2	74	5.1	772.9	57	9.5	27.87	0.04	0.00
39	0	4	4	2	73	4.6	666.7	24.4	4.1	19.08	0.03	0.00
39	1	4	4	1	81	5.6	504.0	89.4	14.9	31.02	0.05	0.00
39	1	4	3	1	86	5.2	425.7	61	10.2	25.59	0.04	0.00

39	1	4	2	1	85	6.4	403.5	78.9	13.2	51.66	0.09	0.00
39	1	4	3	2	84	6.2	452.8	86.7	14.5	43.81	0.05	0.00
39	1	4	1	1	84	6.2	480.7	86.9	14.5	42.91	0.07	0.00
39	1	4	1	2	83	5.6	575.0	74.9	12.5	31.16	0.07	0.00
39	1	4	2	2	79	4.9	724.6	67.2	11.2	23.89	0.07	0.00
39	1	4	4	2	83	5.2	524.7	73.7	12.3	24.79	0.04	0.00
40	0	1	4	1	63	7	871.3	50.9	8.5	69.96	0.06	0.00
40	0	1	2	1	61	6.3	680.1	75.5	12.6	45.70	0.06	0.00
40	0	1	3	1	66	6.4	674.4	38.3	6.4	47.63	0.08	0.01
40	0	1	1	1	66	6.3	660.3	63.8	10.6	45.23	0.08	0.01
40	0	1	2	2	67	5.3	785.5	56.3	9.4	31.20	0.09	0.01
40	0	1	3	2	61	5.4	706.7	57.2	9.5	30.45	0.09	0.01
40	0	1	1	2	67	5.9	823.6	39.8	6.6	42.83	0.04	0.00
40	0	1	4	2	67	5.6	774.8	50.6	8.4	35.66	0.03	0.00
40	1	1	4	1	65	6.2	605.9	50.9	8.5	42.29	0.02	0.00
40	1	1	3	1	64	6.2	549.3	57.4	9.6	42.00	0.02	0.00
40	1	1	2	1	67	5.3	743.4	46.9	7.8	29.94	0.03	0.00
40	1	1	3	2	63	6.5	614.3	55	9.2	48.66	0.02	0.00
40	1	1	1	1	70	6.2	591.0	64.3	10.7	42.14	0.02	0.00
40	1	1	1	2	64	6.1	606.6	75.5	12.6	40.35	0.02	0.00
40	1	1	2	2	64	6.9	643.4	63.2	10.5	58.36	0.02	0.00
40	1	1	4	2	63	6.6	727.1	51.5	8.6	53.52	0.02	0.00
40	0	2	4	1	74	6	833.8	46.4	9.3	45.18	0.03	0.00
40	0	2	2	1	69	6.4	779.8	93.1	7.8	51.11	0.03	0.00
40	0	2	3	1	68	6	998.4	54.7	7.8	53.51	0.02	0.00
40	0	2	1	1	70	5.9	780.1	85.9	7.2	41.14	0.02	0.00
40	0	2	2	2	68	5.3	601.7	60.9	7.6	26.88	0.05	0.00
40	0	2	3	2	73	5.3	916.9	92.8	7.7	35.99	0.05	0.00
40	0	2	1	2	69	5.9	714.4	62	7.8	38.97	0.05	0.00
40	0	2	4	2	67	6.8	858.3	79.3	6.6	64.10	0.05	0.00
40	1	2	4	1	70	6.5	716.2	90	7.5	50.95	0.05	0.00
40	1	2	3	1	68	6.6	669.9	114.2	9.5	51.86	0.04	0.00
40	1	2	2	1	78	6.4	856.2	106.2	8.9	54.59	0.05	0.00
40	1	2	3	2	68	6.6	632.1	135.2	11.3	51.12	0.06	0.00
40	1	2	1	1	68	7	737.5	122.6	10.2	63.42	0.06	0.00
40	1	2	1	2	68	6.9	669.7	114.3	9.5	58.88	0.06	0.00
40	1	2	2	2	72	5.5	736.0	50.7	4.2	32.81	0.05	0.00
40	1	2	4	2	66	6.4	581.9	113.4	9.5	46.23	0.05	0.00
40	0	3	4	1	76	5.6	801.3	31.6	5.3	36.58	0.85	0.05
40	0	3	2	1	80	4.9	586.0	41.2	6.9	21.33	1.12	0.07
40	0	3	3	1	67	4.1	641.1	32.1	5.4	13.67	1.07	0.06
40	0	3	1	1	75	5.5	678.8	34.2	5.7	31.31	1.21	0.07
40	0	3	2	2	75	4.2	809.2	31	5.2	17.75	0.97	0.05
40	0	3	3	2	73	5.4	713.6	35.3	5.9	30.63	0.76	0.04
40	0	3	1	2	68	4.2	700.3	24.8	4.1	15.55	1.23	0.07
40	0	3	4	2	74	5	772.0	46.9	7.8	26.45	1.11	0.07
40	1	3	4	1	74	4.9	557.5	33.8	5.6	21.02	1.12	0.07
40	1	3	3	1	75	5.6	462.2	38.3	6.4	31.51	1.06	0.06
40	1	3	2	1	74	5.4	668.1	33.7	5.6	29.54	1.23	0.08
40	1	3	3	2	75	6	518.2	28.9	4.8	38.18	0.79	0.05
40	1	3	1	1	73	5.2	546.7	34	5.7	24.90	1.11	0.06

40	1	3	1	2	72	5.2	557.6	28	4.7	24.97	0.92	0.06
40	1	3	2	2	72	5	726.3	31.5	5.3	25.24	0.85	0.05
40	1	3	4	2	71	5.5	524.5	27.9	4.7	29.31	0.77	0.05
40	0	4	4	1	85	6.4	726.0	74	12.3	49.12	0.86	0.05
40	0	4	2	1	82	6.2	770.6	65	10.8	46.59	0.82	0.05
40	0	4	3	1	80	5.8	669.1	70.4	11.7	36.02	1.49	0.10
40	0	4	1	1	82	6.6	589.1	79.6	13.3	50.69	1.10	0.08
40	0	4	2	2	84	5	589.2	65.9	11.0	22.62	1.34	0.10
40	0	4	3	2	84	6.2	710.6	75	12.5	44.54	1.65	0.11
40	0	4	1	2	77	5.6	726.7	52.9	8.8	34.16	1.76	0.11
40	0	4	4	2	77	6	850.9	54.9	9.2	45.93	1.27	0.08
40	1	4	4	1	82	5.8	602.1	96.7	16.1	34.81	0.93	0.05
40	1	4	3	1	85	5.5	498.7	69.4	11.6	29.39	0.72	0.03
40	1	4	2	1	86	6.7	630.9	99.3	16.6	53.39	0.86	0.04
40	1	4	3	2	84	6.3	473.7	89.3	14.9	45.34	0.70	0.03
40	1	4	1	1	83	5.9	514.3	85.9	14.3	36.29	0.93	0.05
40	1	4	1	2	83	5.7	610.9	74.9	12.5	33.23	0.48	0.02
40	1	4	2	2	83	5.2	645.7	81.1	13.5	26.18	1.06	0.06
40	1	4	4	2	80	5.7	510.6	69.7	11.6	32.70	1.04	0.06
41	0	1	4	1	64	6.1	866.7	51.2	8.5	48.68	1.49	0.08
41	0	1	2	1	66	6.1	632.4	71.8	12.0	40.73	1.30	0.08
41	0	1	3	1	67	5	919.0	49.6	8.3	31.24	1.19	0.07
41	0	1	1	1	62	6.2	844.0	57.6	9.6	49.69	1.22	0.05
41	0	1	2	2	63	5.2	858.5	47.8	8.0	32.13	1.22	0.06
41	0	1	3	2	67	5.9	794.3	65.7	11.0	41.67	1.49	0.08
41	0	1	1	2	74	6.1	810.0	46.4	7.7	46.15	1.42	0.08
41	0	1	4	2	63	6.1	753.0	46.6	7.8	43.96	1.34	0.08
41	1	1	4	1	65	6	623.3	38.5	6.4	38.70	1.16	0.06
41	1	1	3	1	65	5.6	775.6	76.3	12.7	35.69	0.91	0.05
41	1	1	2	1	71	4.6	822.1	58.2	9.7	22.63	1.06	0.06
41	1	1	3	2	60	6	710.7	69	11.5	40.70	1.46	0.09
41	1	1	1	1	63	6.5	1016.5	64.7	10.8	66.46	1.46	0.09
41	1	1	1	2	66	5.6	712.0	64.7	10.8	33.75	1.35	0.08
41	1	1	2	2	65	6.2	817.3	78.5	13.1	48.49	1.16	0.07
41	1	1	4	2	63	5.8	750.6	57.1	9.5	38.31	1.02	0.06
41	0	2	4	1	72	6.4	1083.7	96.5	8.0	68.62	0.89	0.05
41	0	2	2	1	74	6.3	824.1	87.8	7.3	50.88	1.08	0.06
41	0	2	3	1	71	5.6	867.2	69.3	5.8	39.13	0.90	0.05
41	0	2	1	1	69	6.2	880.8	93.6	7.8	51.46	1.03	0.06
41	0	2	2	2	79	6.5	1063.9	98.8	8.2	69.80	0.83	0.04
41	0	2	3	2	73	5.1	791.9	87.9	7.3	28.43	0.80	0.04
41	0	2	1	2	71	6.1	840.7	91.7	7.6	47.48	0.99	0.05
41	0	2	4	2	63	5.8	868.7	89.7	7.5	42.85	1.18	0.06
41	1	2	4	1	67	5.6	734.1	73	6.1	34.38	1.64	0.08
41	1	2	3	1	69	6.1	730.6	122.4	10.2	43.21	1.23	0.07
41	1	2	2	1	76	6.4	723.2	114.9	9.6	49.03	1.23	0.07
41	1	2	3	2	70	6.1	591.4	113.8	9.5	40.18	1.02	0.06
41	1	2	1	1	72	6.3	1107.9	112.4	9.4	67.77	0.90	0.05
41	1	2	1	2	68	6.3	655.2	123.2	10.3	45.12	0.54	0.03
41	1	2	2	2	72	6.4	772.3	105.6	8.8	50.81	0.67	0.04
41	1	2	4	2	68	6.4	654.8	139.9	11.7	47.18	0.52	0.03

41	0	3	4	1	77	5.5	811.3	34.1	5.7	35.26	0.68	0.04
41	0	3	2	1	78	4.8	568.6	35.9	6.0	19.92	0.60	0.03
41	0	3	3	1	74	4.7	675.8	35.1	5.9	20.39	1.23	0.07
41	0	3	1	1	77	4.9	731.9	39.6	6.6	24.06	1.15	0.07
41	0	3	2	2	78	4.6	854.7	37.4	6.2	23.54	1.57	0.10
41	0	3	3	2	75	5	515.6	30.4	5.1	22.05	1.42	0.10
41	0	3	1	2	73	4.7	756.3	35.6	5.9	22.16	1.19	0.08
41	0	3	4	2	73	3.5	635.7	29.3	4.9	8.96	0.82	0.06
41	1	3	4	1	74	4.4	573.5	31	5.2	15.62	0.95	0.05
41	1	3	3	1	74	6.6	966.7	34.7	5.8	65.66	0.65	0.03
41	1	3	2	1	73	5.4	680.2	33.8	5.6	29.81	0.60	0.03
41	1	3	3	2	76	4.7	495.9	34.3	5.7	18.30	0.53	0.02
41	1	3	1	1	73	4.6	678.9	37.9	6.3	19.31	0.86	0.04
41	1	3	1	2	73	4.1	720.8	26.4	4.4	14.96	0.34	0.01
41	1	3	2	2	73	5.1	639.6	22.3	3.7	24.70	0.87	0.04
41	1	3	4	2	69	5.1	713.2	34.1	5.7	26.27	1.00	0.05
41	0	4	4	1	86	6.6	878.4	73.8	12.3	60.35	1.02	0.05
41	0	4	2	1	84	6.2	832.3	60.4	10.1	49.15	1.04	0.05
41	0	4	3	1	82	5.4	740.0	63.3	10.6	31.36	0.69	0.04
41	0	4	1	1	85	6.1	673.9	80.7	13.5	41.60	0.64	0.03
41	0	4	2	2	85	5.5	606.2	71.4	11.9	29.94	0.65	0.03
41	0	4	3	2	83	5.9	765.4	72.3	12.1	40.61	1.09	0.06
41	0	4	1	2	73	5.2	668.8	53.5	8.9	26.64	0.86	0.05
41	0	4	4	2	78	4.7	656.7	49.6	8.3	20.03	0.87	0.05
41	1	4	4	1	83	5.8	816.9	93.9	15.7	40.70	1.24	0.06
41	1	4	3	1	83	5.7	505.3	80.4	13.4	32.73	0.34	0.02
41	1	4	2	1	87	6.2	648.6	77.1	12.9	42.98	1.24	0.06
41	1	4	3	2	88	5.7	493.2	84.9	14.2	32.84	1.56	0.08
41	1	4	1	1	82	5.8	580.3	89.1	14.9	34.57	1.17	0.07
41	1	4	1	2	82	5.7	782.0	77.4	12.9	37.62	1.27	0.07
41	1	4	2	2	84	5.6	624.5	76.6	12.8	31.80	0.91	0.05
41	1	4	4	2	85	5.3	519.7	76.8	12.8	26.23	0.87	0.05
42	0	1	4	1	58	7.3	925.6	57.9	9.7	81.80	1.11	0.07
42	0	1	2	1	63	6.1	643.3	67.4	11.2	40.93	1.02	0.06
42	0	1	3	1	65	6.1	709.3	52.2	8.7	42.55	1.20	0.07
42	0	1	1	1	66	6.2	677.9	74.9	12.5	43.64	1.05	0.06
42	0	1	2	2	70	5.2	793.8	54.2	9.0	29.95	0.88	0.05
42	0	1	3	2	72	5.8	766.6	57.9	9.7	38.85	0.98	0.05
42	0	1	1	2	60	5.2	814.8	40.7	6.8	30.63	1.60	0.09
42	0	1	4	2	68	5.9	941.2	49.8	8.3	48.27	1.46	0.09
42	1	1	4	1	64	6.2	751.3	52.2	8.7	45.88	2.22	0.13
42	1	1	3	1	63	6.3	681.2	64.3	10.7	45.73	2.20	0.15
42	1	1	2	1	72	4.9	751.3	49.9	8.3	24.55	1.53	0.10
42	1	1	3	2	71	6.3	602.4	64.5	10.8	44.28	1.51	0.09
42	1	1	1	1	66	6.8	747.9	54.9	9.2	58.91	1.00	0.06
42	1	1	1	2	65	5.7	766.9	121.6	20.3	37.10	0.67	0.04
42	1	1	2	2	72	5.9	772.8	66.4	11.1	40.88	0.98	0.06
42	1	1	4	2	64	5.8	763.7	54.7	9.1	38.75	0.84	0.05
42	0	2	4	1	73	6.3	836.6	71	8.9	51.46	0.93	0.06
42	0	2	2	1	72	6.4	642.4	106.7	8.9	46.94	0.74	0.04
42	0	2	3	1	70	6	1028.1	94.7	7.9	55.25	1.83	0.12

42	0	2	1	1	72	6.6	872.8	103.8	8.7	60.05	1.72	0.11
42	0	2	2	2	72	5.7	752.5	52.1	4.3	36.63	1.60	0.11
42	0	2	3	2	71	6.2	819.3	90.3	7.5	48.57	1.76	0.13
42	0	2	1	2	75	5.1	878.0	91.3	7.6	31.28	1.37	0.09
42	0	2	4	2	65	6	714.0	104.5	8.7	40.80	1.35	0.09
42	1	2	4	1	67	6.2	800.8	90.4	7.5	47.79	1.02	0.06
42	1	2	3	1	66	6.4	716.1	120.7	10.1	48.81	0.68	0.03
42	1	2	2	1	73	6.9	887.5	111.6	9.3	68.31	0.71	0.04
42	1	2	3	2	70	6.3	612.6	134.5	11.2	44.40	0.62	0.03
42	1	2	1	1	64	6.8	716.0	130.3	10.9	57.77	1.27	0.06
42	1	2	1	2	71	6.5	662.9	51	4.3	49.50	0.50	0.03
42	1	2	2	2	71	5.3	777.9	45.9	7.7	30.96	1.15	0.06
42	1	2	4	2	66	6.9	668.3	141.8	11.8	58.85	1.23	0.08
42	0	3	4	1	78	5.5	857.4	36.7	6.1	36.99	1.66	0.10
42	0	3	2	1	73	4.9	603.7	32.8	5.5	21.56	1.40	0.09
42	0	3	3	1	73	4.7	718.7	33.5	5.6	21.28	1.06	0.05
42	0	3	1	1	78	4.5	548.2	38.9	6.5	16.39	0.78	0.04
42	0	3	2	2	75	5.1	825.7	33.9	5.7	29.49	0.90	0.05
42	0	3	3	2	73	4.9	659.5	27.8	4.6	22.48	1.28	0.07
42	0	3	1	2	74	3.9	682.5	29.6	4.9	12.56	1.60	0.09
42	0	3	4	2	75	5.2	807.2	40.9	6.8	30.38	1.41	0.08
42	1	3	4	1	72	5	697.0	38	6.3	24.55	1.09	0.06
42	1	3	3	1	75	5.6	471.3	29.2	4.9	31.36	0.72	0.04
42	1	3	2	1	71	4.5	608.0	30.3	5.1	17.07	1.08	0.06
42	1	3	3	2	74	5.8	600.4	38.7	6.5	34.79	1.94	0.12
42	1	3	1	1	76	4.8	653.1	37.9	6.3	21.14	1.82	0.12
42	1	3	1	2	70	4.2	686.3	25.2	4.2	15.30	1.51	0.09
42	1	3	2	2	75	4.5	870.3	28.7	4.8	22.73	1.39	0.08
42	1	3	4	2	73	4.3	780.0	32.9	5.5	18.15	1.17	0.07
42	0	4	4	1	84	7.3	843.4	67.5	11.3	76.46	0.54	0.03
42	0	4	2	1	85	6.2	718.8	74.3	12.4	44.79	0.48	0.03
42	0	4	3	1	81	5.7	634.5	65.1	10.9	33.61	0.60	0.04
42	0	4	1	1	82	6.2	552.2	68.7	11.5	42.00	0.53	0.03
42	0	4	2	2	80	5.2	581.9	69.6	11.6	25.21	0.40	0.02
42	0	4	3	2	67	5.8	676.4	74	12.3	36.20	0.46	0.03
42	0	4	1	2	76	5.1	892.0	52.7	8.8	31.79	1.17	0.06
42	0	4	4	2	78	5.7	753.6	55.5	9.3	36.66	0.84	0.05
42	1	4	4	1	81	6	606.6	91.5	15.3	38.46	0.94	0.06
42	1	4	3	1	84	5.4	485.3	70.6	11.8	27.87	0.99	0.07
42	1	4	2	1	87	6.3	617.2	80.5	13.4	44.46	0.82	0.05
42	1	4	3	2	85	6.1	500.8	85.9	14.3	40.38	0.77	0.05
42	1	4	1	1	82	6.5	502.6	85.5	14.3	49.16	0.43	0.03
42	1	4	1	2	79	5.4	609.7	68.1	11.4	28.46	0.31	0.02
42	1	4	2	2	80	5.4	645.7	64.9	10.8	29.08	0.44	0.03

Table B2. The yield of tomato varieties grown with and without additional light and treated with three different nutrient solutions at Bioforsk Vest. Særheim in 2011.

Light	Type	Treatment	No. of fruits harvested pr m2	Yield kg/m2	SSC Yield kg /m2
0	1	2	707.0	9	0.4
0	1	3	992.0	9	0.3
0	1	1	714.0	10	0.4
0	1	2	707.0	9	0.4
0	1	3	611.9	8	0.3
0	1	1	665.0	8	0.3
1	1	3	1214.7	15	0.3
1	1	2	1283.9	15	0.2
1	1	3	1297.9	16	0.3
1	1	1	1428.7	17	0.3
1	1	1	1195.8	15	0.3
1	1	2	1256.6	15	0.3
0	2	2	725.9	9	0.2
0	2	3	573.4	7	0.1
0	2	1	625.2	8	0.3
0	2	2	628.7	7	0.2
0	2	3	670.6	7	0.2
0	2	1	652.4	7	0.3
1	2	3	1329.4	15	0.5
1	2	2	1404.2	16	0.2
1	2	3	1258.0	16	0.3
1	2	1	1414.0	16	0.4
1	2	1	1288.1	15	0.4
1	2	2	1120.3	12	0.4
0	3	2	879.0	9	0.7
0	3	3	718.9	6	0.6
0	3	1	771.3	7	0.5
0	3	2	760.8	6	0.6
0	3	3	844.8	8	0.6
0	3	1	655.9	5	0.6
1	3	3	1321.0	11	0.7
1	3	2	1315.4	10	0.7
1	3	3	1516.8	12	0.7
1	3	1	1460.8	11	0.5
1	3	1	1273.4	10	0.7
1	3	2	1207.7	9	0.7
0	4	2	546.9	8	0.4
0	4	3	551.0	9	0.3
0	4	1	611.2	11	0.4
0	4	2	562.9	10	0.3
0	4	3	530.1	8	0.4
0	4	1	443.4	6	0.4
1	4	3	858.7	13	0.6
1	4	2	924.5	16	0.4
1	4	3	918.9	16	0.7
1	4	1	899.3	15	0.4
1	4	1	786.0	13	0.5