JALOVEC, JEFFREY SCOTT Ph.D. Exploring the Effects of Fluorophore Additives and Narrow Band Light on Photosynthetic Organisms. (2022)
Directed by Dr. Daniel Herr. 120 pp.

The research undertaken here explores the effects of fluorescent sensitizers on photosynthetic organisms. Further, the research explores the effect of narrow band light environments on photosynthetic cyanobacteria. This work elucidates the role of light frequency on photosynthetically driven biomass generation. We hypothesized that the introduction of fluorescent sensitizers, which convert unused portions of the visible spectrum to photosynthetically useful light, would enhance the organism's growth rates and biomass. We further hypothesized that the cyanobacteria grown in narrow band light environments would undergo a genomic response and induce a genetic response that stimulated an increase in various accessory pigments more suitable to absorbing the supplied wavelengths.

The first set of studies involved adding a trans-stilbene dye, as a fluorescent sensitizer that absorbs UV light and emits blue, to *Linum usitatissimum* (Golden Flax). We used a hydroponic delivery system to create our closed loop nutrient stream and supply the sensitizer. We hypothesized that the experimental group would grow faster and generate more biomass due to additional photosynthetically useful light being available. We found that the height of the population given the sensitizer increased by 43% under normal grow lights and when additional UV light was supplied the additional height was increased to 77%. The biomass of the crop portion of the experimental group was greater. However, we did note that the roots and leaves of the experimental group were smaller than the control group. This led us to discover that the overall biomass showed no statistical difference between the groups. The control group weighed 4.90±.87 grams compared to the test groups 4.92±.58 grams. Through optical density readings of

the supplied microbiome grown in the presence of the sensitizer we determined that there was no deleterious effect on the overall growth rate.

The second set of experiments we conducted involved the addition of the optical sensitizer to the growth media of the cyanobacteria Synechococcus elongatus. We hypothesized that the experimental group would grow faster and generate more biomass due to additional photosynthetically useful light being available. We inoculated flasks with Synechococcus elongatus and monitored their growth through optical density readings. The growth of the experimental group showed a marked increase as the flasks became saturated. We also performed genomic testing on the populations after continual growth in the presence of the sensitizer for 8 weeks. We found no noteworthy genomic changes in Synechococcus elongatus due to the presence of the sensitizer in the growth media. This implies the sensitizer is not mutagenic for our organism.

The final set of experiments involved the growth of Synechococcus elongatus in narrow band light environments. We hypothesized that the cyanobacteria would mutate to accommodate the light environments and increase or decrease the concentrations of various pigments as necessary to thrive. For this study we used full spectrum light for a control, orange light and green light. In the early stages the control group outgrew the experimental groups handily. However, over time the experimental groups grew at a comparable pace to the control. We performed UPLC analysis on the samples but found no significant fluctuations in the concentrations of photosynthetic pigments. Further, upon genomic sequencing we found no noteworthy changes to the genomes of the cyanobacteria that experienced various narrow band light treatments.

EXPLORING THE EFFECTS OF FLUOROPHORE ADDITIVES AND NARROW BAND LIGHT ON PHOTOSYNTHETIC ORGANISMS

by

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A Dissertation

Submitted to

the Faculty of The Graduate School at

The University of North Carolina at Greensboro

in Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

Greensboro

2022

Approved by

Dr. Daniel Herr
Committee Chair

DEDICATION

I dedicate this dissertation to my family. My mother, Jeanne Case, for all her guidance and patience through the years. My stepfather, Bob Case, for teaching me the meaning of selflessness and dedication. My father, Steve Jalovec, for always being available to offer advice and encouragement. My daughters, Loren and Meredith Jalovec, for giving me a reason to strive and to achieve. I hope I can be an example of what hard work can eventually lead to. Finally, to my wife, Jillian Jalovec. I can't summarize all the vital support you've shown throughout this process. Even when things didn't go according to plan, your understanding and love have been a beacon for me when I've lost my way. I love you all and can't express enough my gratitude and the overwhelming love I feel from, and for, all of you. Your unwavering support and belief in me, through all the ups and downs, has led me to become the man I am today. From the bottom of my heart, I dedicate this to you all.

APPROVAL PAGE

This dissertation written by Jeffrey Scott Jalovec has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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ACKNOWLEDGEMENTS

I would like to acknowledge my committee, Dr. Daniel Herr, Dr. Joseph Graves, Dr. Denis LaJeunesse, and Dr. Jianjun Wei for their guidance and patience as I completed this work. I would also like to acknowledge Dr. Akamu Jude Ewankum, Dr. Misty Thomas, and Mr. Tyler Graf for their assistance in the lab as well as their input and advice on experimentation. I could not have done this without you.

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CHAPTER I: INTRODUCTION

This research provides strategies for mimicking and increasing the effects of accessory pigments on photosynthetic organism growth. We accomplished this through the addition of fluorescent compounds that emit in the photosynthetically useful region and exploring the evolutionary response of photosynthetic organisms to narrow band light environments. The overarching goal of these experiments will lead to understanding mechanisms for increasing the overall growth rates of these organisms through the addition of fluorescent compounds and through increased accessory pigment production from evolutionary response to long term growth in limited light environments.

1.1 Photosynthesis

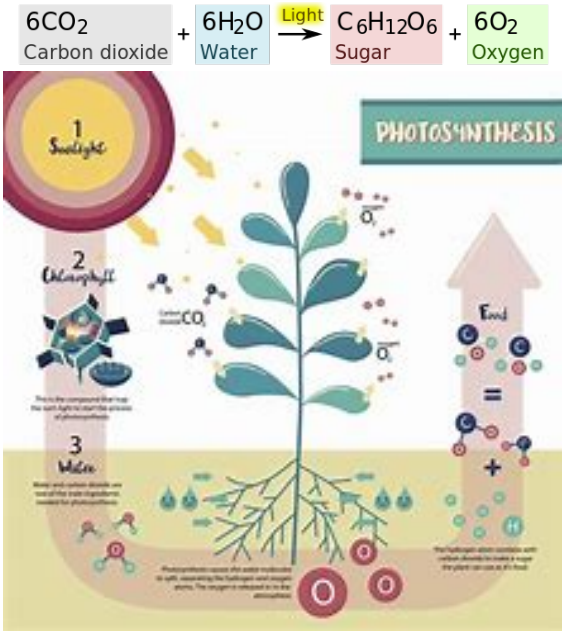
Most plants, algae, and cyanobacteria, also known as photoautotrophs, perform photosynthesis to supply the energy they need to grow and proliferate. All other living organisms on the planet ingest this photosynthetically produced energy, directly or indirectly, and use it to survive. (23) In this photochemical process, photoautotrophs convert light energy from the sun into chemical energy that can be stored as carbohydrate molecules, such as sugars, and be used later by the organism to perform its' activities. (1) The reaction involves converting carbon dioxide and water into sugars with a waste product of oxygen. Therefore, we recognize photosynthesis as the primary process for generating and maintaining the oxygen levels in the atmosphere and for sequestering atmospheric carbon dioxide. (2)

The overall equation of photosynthesis, $6CO_2 + 6H_2O + \mathrm{hv} \rightarrow C_6H_{12}O_6 + 6O_2$, where hv is electromagnetic radiation energy, is a widely used simplification of the complex series of chemical reactions that constitute photosynthesis. The process has been divided into two

categories of reactions, the light cycle and the dark cycle. The light cycle reactions can only occur when there is electromagnetic radiation supplied to the organism typically light from the sun or an artificial light source. Whereas the reactions of the dark cycle do not require the input of external energy to occur. (79) The research conducted here focuses exclusively on light cycle reactions. Specifically, the critical step of absorption of photons by pigments.

Though exact photosynthetic mechanisms and activity can vary from species to species, they always instigate the chemical reaction through the absorption of energy from incident light by pigments contained within the organisms. Plants and most other photosynthetic organisms, except for some photosynthetic bacteria, use Chlorophyll-a as the primary pigment for photosynthesis and it is the only pigment that can convert light energy to chemical energy directly. (78)

Figure 1. Photosynthesis Diagram and Chemical Equation

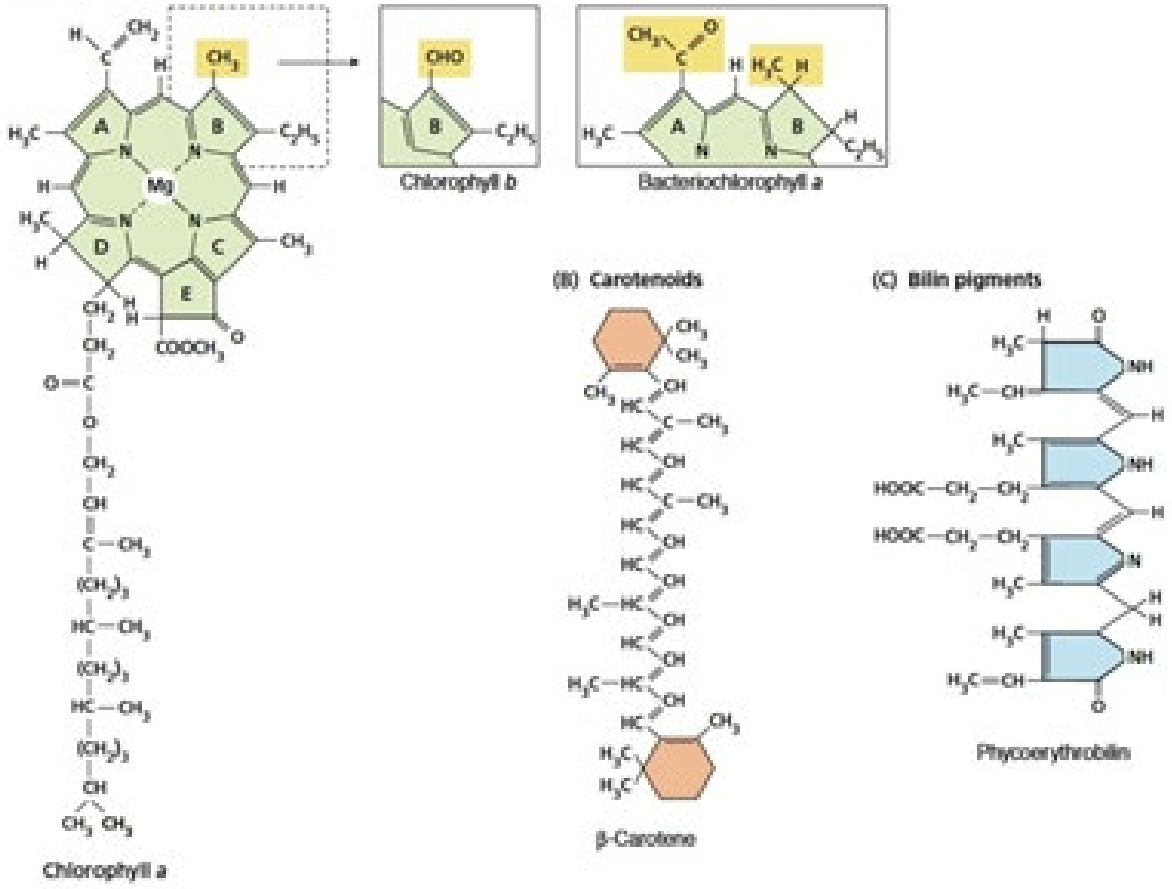


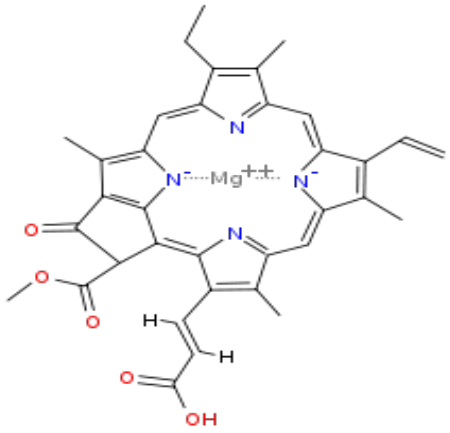
Note. Sourced from Wikimedia Commons

Chlorophyll-a has the composition $C_{55}H_{72}O_5N_4Mg$ and exhibits a green visual color with absorption peaks at 430nm and 662nm. Photoautotrophs utilize accessory pigments to shift energy to Chlorophyll-a because it absorbs a narrow range of energies.

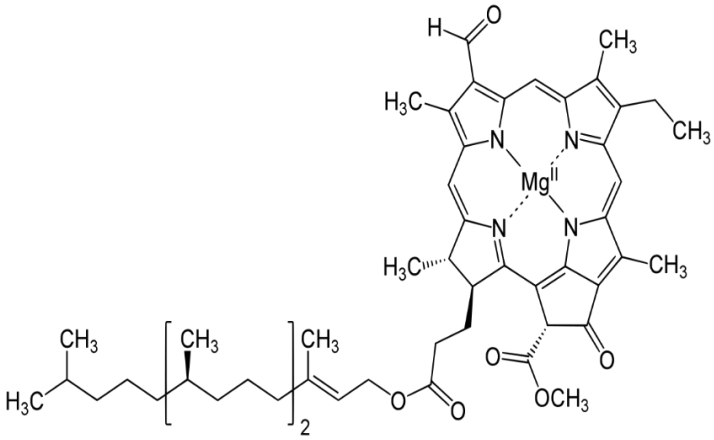
The primary accessory pigment, chlorophyll-b, has the composition $C_{55}H_{70}O_6N_4Mg$. The difference from chlorophyll-a being the replacement of a methyl group with a CHO group. It exhibits a blue-green visual color with absorption peaks at 453nm and 642nm. It occurs in all plants, green algae, and some prokaryotes. Usually, plants contain about half as much chlorophyll- b as the -a variety. (2,3) Many other accessory pigments exist that assist with the transfer of energy

Figure 2. Chemical Structure of Chlorophyll and Accessory Pigments





Chlorophyll-c

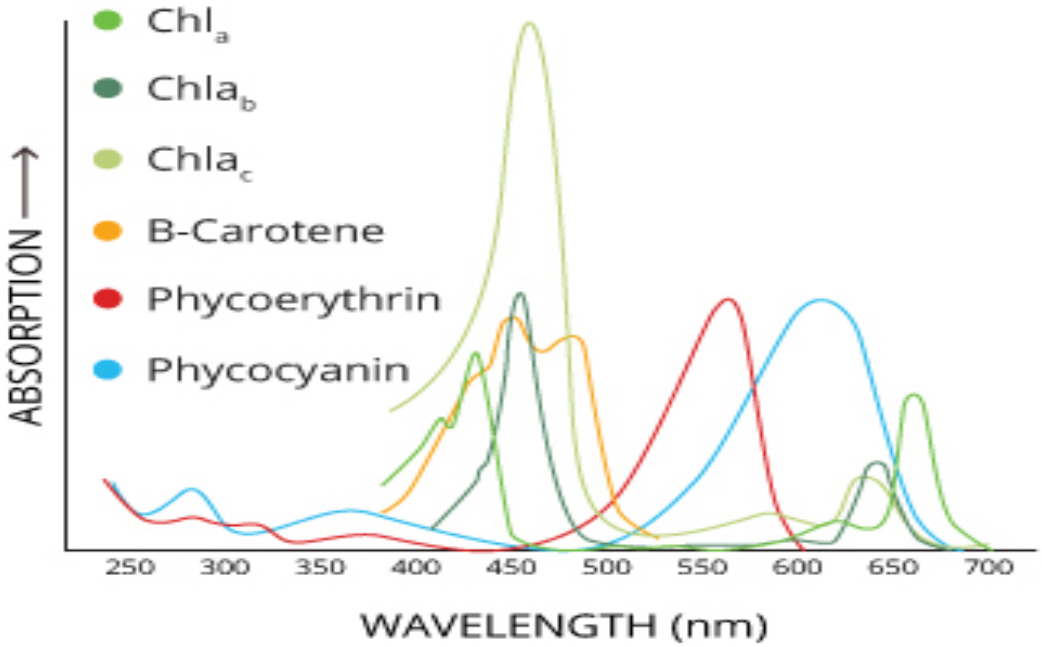


Chlorophyll d

Note. Modified from image sourced from http://www.tankonyvtar.hu/

to Chlorophyll-a including Chlorophyll-c, Chlorophyll-d, carotenoids, and phycobiliproteins. (4) They all have slightly different absorption spectra yielding a much broader composite absorption spectra for the photosystem and can therefore utilize many wavelengths of light.

Figure 3. Composite absorption spectra of photosynthetic pigments.



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The various pigments arrange themselves into light harvesting antennae which are far more efficient at absorbing light energy than the individual pigments alone. As the pigments absorb light, electrons in the molecules are excited to higher energy states. Next, a series of enzymes known as electron transport chains, funnel the energy supplied to these electrons into reactions that store the energy in chemical bonds. (79) During this process, two high energy molecules, namely NADPH and ATP, are produced from $NADP^+$ and ADP, respectively. NADPH and ATP supply the energy necessary for the dark cycle to produce carbohydrates from the fixing of carbon supplied by CO_2. (79)

As light energy from the sun travels through the Earth's atmosphere and through water, the available wavelengths of energy get absorbed. The accessory pigments have evolved to utilize the most abundant wavelengths that make it through to their locations.

Figure 4. Solar Spectrum That Reaches Various Levels of Earth.

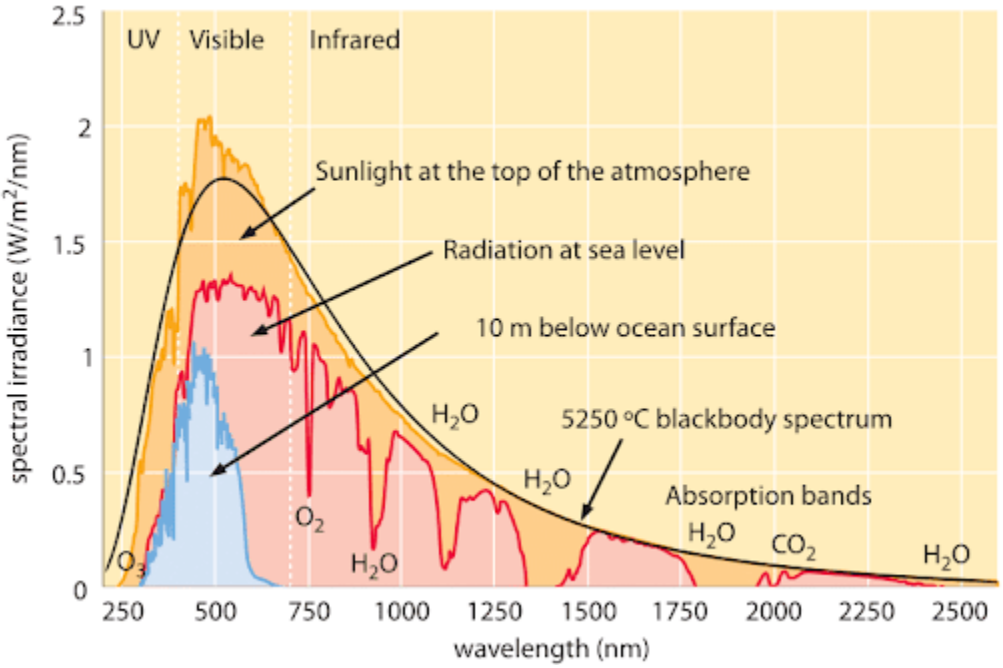

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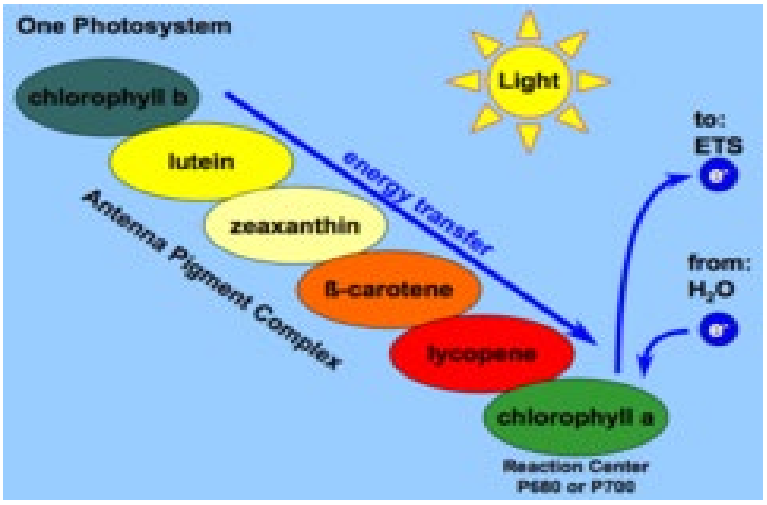
Figure 5. Energy Transfer Mechanism of Accessory Pigments to Chlorophyll a



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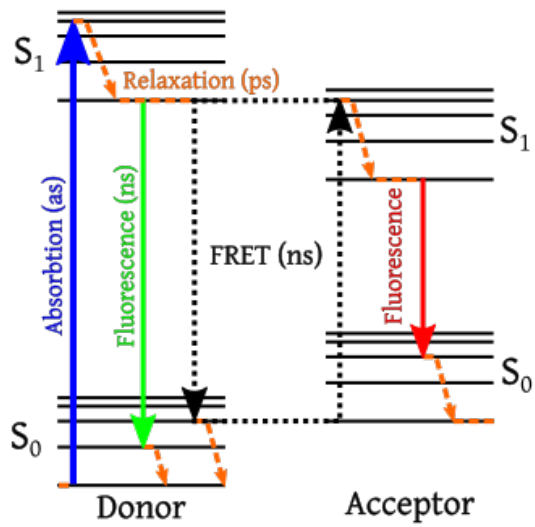
1.2 Electron Transport Chain and Forster Resonance Energy Transfer (FRET)

Many chemical reactions require specific energies to activate them including many photochemical reactions. (5) Electromagnetic radiation has an energy associated with it that increases as the wavelength of the radiation decreases. (6) Photosynthesis activation energy corresponds with the absorption peaks of Chlorophyll-a, i.e., the energy of red and blue light. This means other wavelengths of light only become photosynthetically useful when converted to the appropriate energy through fluorescent compounds or when accessory pigments transfer the absorbed energy through FRET.

Fluorescence, the emission of a photon, occurs when a molecule that has absorbed energy from electromagnetic radiation or some other method returns to its ground state. The radiation excites the molecule by raising an orbital electron to its singlet state and as the molecule relaxes

the electron returns to its ground state while emitting a photon with energy equal to the difference in energy levels between the electronic energy states. (7) The phenomenon known as Stokes shift

Figure 6. Jablonski Diagram of Fluorescence and FRET, with Typical Timescales



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states that the emitted photon generally has a longer wavelength, and therefore lower energy, than the absorbed radiation due to energy loss, typically as heat, between the initial photon absorption and the emission of the photon. (8) However, with intense enough absorbed electromagnetic radiation, it becomes possible for one electron to absorb two photons. Two-photon absorption can lead to emission of radiation having a shorter wavelength than the absorbed radiation. The emitted radiation may also be of the same wavelength as the absorbed radiation, termed "resonance fluorescence". (7,8) Specifically selected fluorophores can absorb non-photosynthetically useful light and the emitted photons can, in turn, supply the appropriate activation energy.

Forster resonance energy transfer, a mechanism named for German scientist Theodor Forster, describes nonradiative energy transfer between two chromophores, i.e., light sensitive molecules. (9) The electron transport chain of photosynthesis relies on FRET to transfer light

energy absorbed by accessory pigments to Chlorophyll-a. (12) FRET efficiency being inversely proportional to the sixth power of the distance between the donor and acceptor molecules makes the process highly sensitive to even the smallest changes in distance between them. (10) The process begins with the excitation of a donor chromophore to an excited electronic state. The energy absorbed by the donor can then be transferred to the acceptor molecule through nonradiative dipole-dipole coupling. (11)

1.3 Evolutionary Biology

Evolution, being one of the central unifying concepts of biology, seeks to offer a comprehensive explanation for the patterns of similarities and differences that exist in all species. (13) Charles Darwin laid out the basic tenets of evolutionary theory in "The Origin of Species" where he detailed his core theory of the principle of natural selection. This principle states that organisms that adapt to their environment become more likely to survive and leave more offspring. Modern biology has been shaped by this concept and it has led to the development of many subfields of biology including experimental evolution which makes use of experiments or controlled field manipulations to explore evolutionary dynamics. (14) Due to the large number of generations required to see adaptions, evolution experiments generally utilize microorganisms such as bacteria, viruses, and yeast because of their rapid generation times. (14-16) As knowledge and technology have improved it has become possible to identify mutations that occurred during adaptation to environmental stimuli using whole genome sequencing, known as Evolve and Resequence. (17-19) This type of experimentation has been performed on viruses, prokaryotes, and both multicellular and unicellular eukaryotes. (19-22)

The relatively new field of evolutionary biology only recently gained prominence with most universities not having departments of evolutionary biology until the 1980's when areas such as molecular and cell biology and ecology replaced older departments such as botany and zoology. Microbiology has become increasingly important to the evolutionary discipline as microbial physiology and genomics become better understood. The rapid generation time of bacteria and viruses and increasing understanding of genetic architecture has made it possible to explore many evolutionary questions including adaptation and speciation. (62-65) Evolutionary forces include natural selection, sexual selection, genetic drift, developmental constraints, and mutation bias and current research endeavors to determine their relative importance. (66) Natural selection, the process whereby organisms better adapted to their environment tend to survive and produce more offspring, is the primary evolutionary force with respect to these experiments.

One of the most widely known and longest running, in terms of generations, experiments of laboratory bacterial evolution is Richard Lenskis' *E. Coli* experiment in which one of twelve lineages, under identical growth conditions, developed the ability to aerobically metabolize citrate from the media and showed greatly increased growth rates. (67-69) Several shorter experiments currently used in classrooms teach evolutionary concepts from resistance to antibiotics to multicellularity evolution. (70,71) Improved technology and understanding opens the possibility of conducting the experiments, sequencing the evolved genomes, and analyzing and interpreting the results in a classroom setting. (72)

1.4 State of the Art Methods to Increase Photosynthetic Activity and Growth Rates

The process of photosynthesis has long been evolving and this led to much debate over whether the efficacy could be improved. (23, 24) Vast evidence exists that indicates

photosynthesis is ancient, originating not long after life itself, and has evolved along a complex path yielding the wide variety of photosynthetic organisms and metabolisms present today. (23-25) Photosynthetic ability has been found to be widely distributed in the bacterial phyla including cyanobacteria, proteobacteria, green sulfur bacteria, firmicutes, filamentous anoxygenic phototrophs, and acidobacteria, and has been found in Eukarya. (26-28) Compelling evidence shows that eukaryotic photosynthesis arose from endosymbiosis of cyanobacterial-like organisms that eventually evolved into chloroplasts. (29) Therefore, it seems as if the evolutionary origin of photosynthesis can be found in the bacterial domain. Some evidence suggests that photosynthetic organisms existed 3.2-3.5 billion years ago. (25) However, this evidence has not been accepted by all and has led to much debate about when photosynthesis first evolved. (26) Regardless of exactly when photosynthesis first evolved, the process has been occurring for a very long time and improvements have been made by nature through evolution. (30) Further, in nature tradeoffs must be made that effect the efficacy of photosynthesis in photoautotrophs, i.e., for many food crops such as wheat, barley, potatoes, and sugar beets the theoretical maximum conversion of light energy to chemical energy is only 5%. (31,32)

According to Professor Howard Griffiths, Department of Plant Sciences at the University of Cambridge and director of the Combining Algal and Plant Photosynthesis (CAPP) initiative, "Plants really matter, and for the next generation, plant and microbial productivity will become the focus of key global issues: the basis for feeding an additional 2-3 billion mouths, to drive forward an economy currently trading on past sunlight and maintain biodiversity in the face of climate change." (31-33) This initiative represents a collaboration of four transatlantic research teams with the aim of "overcoming limitations in photosynthesis which could then lead to ways of significantly increasing the yield of important crops for food production or sustainable bioenergy."

(31) In most photoautotrophs, growth rates become limited by the rate of CO_2 sequestration and conversion. (32)

Increasing the rates of photosynthesis and growth in photoautotrophs has become increasingly important in recent years for a variety of reasons including increasing crop yields to mitigate food shortages as well as to increase biomass for conversion to fuel. (31-33) Several complementary and parallel aims seek to accomplish this goal. They include increasing the amount of CO_2 available for the organisms, increasing the conversion rate of CO_2 by enzymes in the photosynthetic process to usable sugars, manipulating light exposure and temperature to boost photosynthetic rates, manipulating nonphotochemical quenching mechanisms, and using genetic modification to give organisms with low photosynthetic rates mechanisms employed by organisms with faster rates.

1.4.1 Increased CO_2 Availability and Conversion

Photosynthesis in nature primarily becomes limited by the capture and conversion of CO_2 to usable sugars which occurs during the dark cycle and is therefore not the focus of this research. (31-36) It has been shown that short-term CO_2 enrichment stimulates the rate of photosynthesis and enhances plant mass. (34-37) However, the long-term effects of CO_2 enrichment vary greatly with prolonged exposure reducing the initial stimulation of photosynthesis and suppressing the rate of photosynthesis. (38) This has been attributed to the accumulation of excess carbohydrates in leaves or decreased nitrogen content. (35,37,38) Species that have sink organs for carbohydrates did not show the same suppression of photosynthesis. (38) However, the suppression of photosynthesis always showed an accompanying decrease in nitrogen and Rubisco, an enzyme used in the conversion of CO_2 to carbohydrates, content in the

organisms. (38). Increasing the atmospheric CO_2 concentration significantly increased the maximum quantum efficiency of photosynthesis and improved light energy conversion. (39) Paralleling these results, the leaf-area-based rates of photosynthesis increased under increased CO_2 and became more pronounced in slower growing organisms. (40)

A mechanism known as a Carbon Concentrating Mechanism (CCM) can be found in many algae (32-33). This mechanism increases the concentration of CO_2 in photosynthesizing cells without the need to increase the atmospheric CO_2 allowing Rubisco to operate closer to its maximum efficiency. (32) The CAPP group has done much work in identifying the genes responsible for this mechanism and how it works. (32-33). Upon understanding this mechanism and how the organisms that utilize it handle the increased carbohydrate content and decreased nitrogen content they hope to apply their findings to photoautotrophs universally. (32)

1.4.2 Manipulating Environmental Factors

Many environmental factors, in addition to the atmospheric CO_2 levels, affect the rate of photosynthesis. (41,42) These include ambient temperature, water availability, and lighting conditions. (41-47). Net photosynthesis has an optimization in most plant species at approximately 25ºC. (41) Observations showed a reduction in the overall photosynthetic rates at both higher and lower temperatures attributed mostly to increased dark and photo respiration. (42,43) Comparing the range of temperatures from 10ºC-40ºC at various CO_2 concentrations showed that the rate of photosynthesis still peaked around 25ºC and that the increased temperature did not affect the ability of the organism to sequester CO_2. (40, 44)

The light available to photosynthetic organisms also has a significant effect on their photosynthetic rates. (46,47,48) Under full direct light, organisms tend to become damaged by the

excess energy applied to them in a process known as photobleaching. (48) For this reason, many organisms developed means of protecting themselves from excess radiation by dissipating some light energy as heat in a process known as nonphotochemical quenching (NPQ). (48) When the light reduces to more suitable levels the organism shuts down this process and attempts to gather as much light as possible again. NPQ continues for many minutes after the return to suitable lighting conditions resulting in a loss of photosynthetic efficiency. (48) Through bioengineering, the possibility exists to allow a more rapid return to full photosynthetic operation resulting in increased plant biomass growth by 15%-20%. (48) In natural environments, full direct light does not occur due to atmospheric scattering, but photosynthetic organisms still require NPQ to avoid damage. (46) Under diffuse lighting conditions, such as those on overcast days, higher instantaneous crop photosynthesis rates occur than under direct lighting conditions. This has been attributed to a more homogeneous distribution of light energy over the canopy. Green houses mimic these effects. (46) In controlled environments, the possibility exists of generating highly diffuse light with high irradiance and it has been found that gross photosynthesis increased by 20% under these conditions when compared to direct light application at the same irradiance levels. (47)

1.4.3 Genetic Modification

Through genetic modification, photosynthetic rates show a marked improvement. (30-35) Some organisms have shown enhanced abilities to photosynthesize under various environmental conditions, specifically their ability to sequester atmospheric CO_2 and concentrate it to maximize the efficacy of Rubisco through CCM. The mechanisms involved remain to be characterized and the discovery of the genes responsible through genomic sequencing still must occur. (31-33,35) After determining the genes responsible for CCM in algae and how they work,

the CAPP initiative aspires to transfer the most significant genes into a plant and determine if plants can generate the CCM. If so, they believe that the experimentally created plant will have higher rates of photosynthesis and, therefore, a higher rate of growth than normal plants. (32)

1.5 Characterization of Morphological Bacterial Changes

Generally, bacterial classification occurs by direct examination with optical or scanning electron microscopy to determine their morphology and aggregation. The basic forms consist of coccus (spheres), bacillus (cylinders), coccobacillus (intermediate between spheres and rods), and spiral. (49-54) In addition to these shapes, how they aggregate constitutes another important factor in bacterial morphology. (51) For coccus bacteria, aggregation classifications include diplococci (pairs), tetrads (groups of four), sarcina (micrococci or groups of eight), streptococci (bead like chains), and staphylococci (grape like clusters). (51) For bacillus bacteria, aggregation classifications include diplobacilli (pairs) and streptobacilli (chains). (53) Spiral bacteria also have further classification into spirilla, spirochetes, or vibrios by the number of twists per cell, cell thickness, cell flexibility, and motility. (54)

1.6 Characterization of Morphological Plant Changes

For this research we looked at the vegetative morphology of the plants in response to the presence of a fluorescent sensitizer. The major features we characterized were the height of the stems, the length of the roots, the overall biomass, the number of leaves, the surface area of leaves, and the color and shape of the leaves.

1.7 Characterization of Bacterial Genomic Changes

The characterization of bacterial genomic changes involves the comparison of the genomic sequences from initial growth cultures to late-stage growth cultures. To sequence the genomes, we first extract the DNA from the cultures. Next, the DNA is fragmented and tagged before amplification through polymerase chain reaction (PCR). After amplification, the DNA fragments are pooled and run through a sequencer. The resulting sequences can be compared to look for selective, or hard, sweeps. Selective sweeps are a process where an advantageous mutation increases in frequency in the population. (70)

1.8 Aims and Hypotheses of This Research

This research has three main aims. First, explore the effects of fluorescent sensitizers on the growth and morphology of a model plant species, Golden Flax. We hypothesize that the fluorescent sensitizer will cause the plants to grow faster and generate more biomass due to additional photosynthetically useful light being available without altering the vegetative morphology. Second, explore the effects of fluorescent sensitizers on the growth and morphology of a model cyanobacteria species, *Synechococcus elongatus*. We hypothesize that the fluorescent sensitizer will cause the cyanobacteria to grow faster and generate more biomass due to additional photosynthetically useful light being available without altering the morphology or the genome. Finally, explore the effects of narrow band light environments on the growth and pigment production of a model cyanobacteria, *S. elongatus*. We hypothesize that the cyanobacteria will mutate to accommodate the light environments and increase or decrease the concentrations of various pigments as necessary to thrive. We will explore the up or down regulation of genes involved in the multi-step biosynthetic pathway for production of target

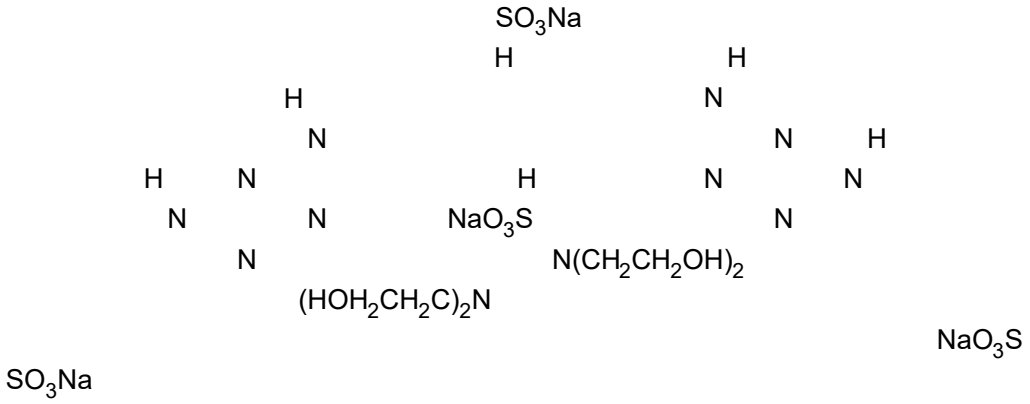
pigments. We will monitor chlG for chlorophyll-a, crtB for beta carotene, cpcB for phycocyanin, and ppC as a housekeeping gene. These genes are responsible for encoding enzymes involved in the production of the target pigments.

CHAPTER II: HYDROPONIC ADDITION OF A FLUOROPHORE TO FLAX PLANTS

2.1 Toward Understanding the Effects of Hydroponic Addition of a Fluorophore to Flax Plants

In this section, we look at the addition of a fluorescent trans-stilbene optical brightener, Day-Glo D282, to hydroponically grown flax plants. We chose D282 as the additive due to its optical properties, as described below, and its reported low level of toxicity to some photosynthetic organisms, e.g., algae.

Figure 7. Chemical Structure of Optical Brightener DayGlo Dye D282.



Note. Drawn with ACD/Labs 2020.1.2 ChemSketch.

The compound absorbs UV light, which can be damaging to photosynthetic organisms, and emits photosynthetically useful blue light. This means that for each quanta of light that is supplied to the organism a greater range of the spectrum can be used. Hence, the addition of such optical brighteners could help to reduce the amount of light, and therefore energy, necessary to grow these organisms in greenhouses or other closed settings where natural light is scarce. Further, in natural light environments, this approach also may contribute to increased

photosynthetic activity while mitigating photoinduced damage due to UV light. We hypothesize that optical fluorescors, such as D282, with emission bands that overlap with the Chlorophyll A absorption spectrum will significantly affect photosynthesis and plant growth rates. We also investigate the macroscopic effect of the D282 on the supplied microbiome of the flax plants by monitoring its growth over time. Based on D282s low toxicity towards algae, we also hypothesize that its impact on the microbiome will correlate with its MSDS toxicity rating.

We predict that the addition of D282 to hydroponically grown flax plants will lead to 1) an increase in the growth rate of the plant crop portion, 2) an increase in the growth rate of the plant root portion, 3) an increase in the leaf surface area and 4) an overall increase in biomass. This effect would be due to the additional photosynthetically useful light supplied to the system by D282 shifting light from the unused UV portion of the spectrum to the more useful blue region, via fluorescence. Also, we predict that the D282 will have no deleterious macroscopic effect on the growth rate of the supplied microbiome, as measured by microbiome growth rates. We think this will be the case because its material safety data sheet states a low level of toxicity to algae observed at much higher concentrations than those used in this study.

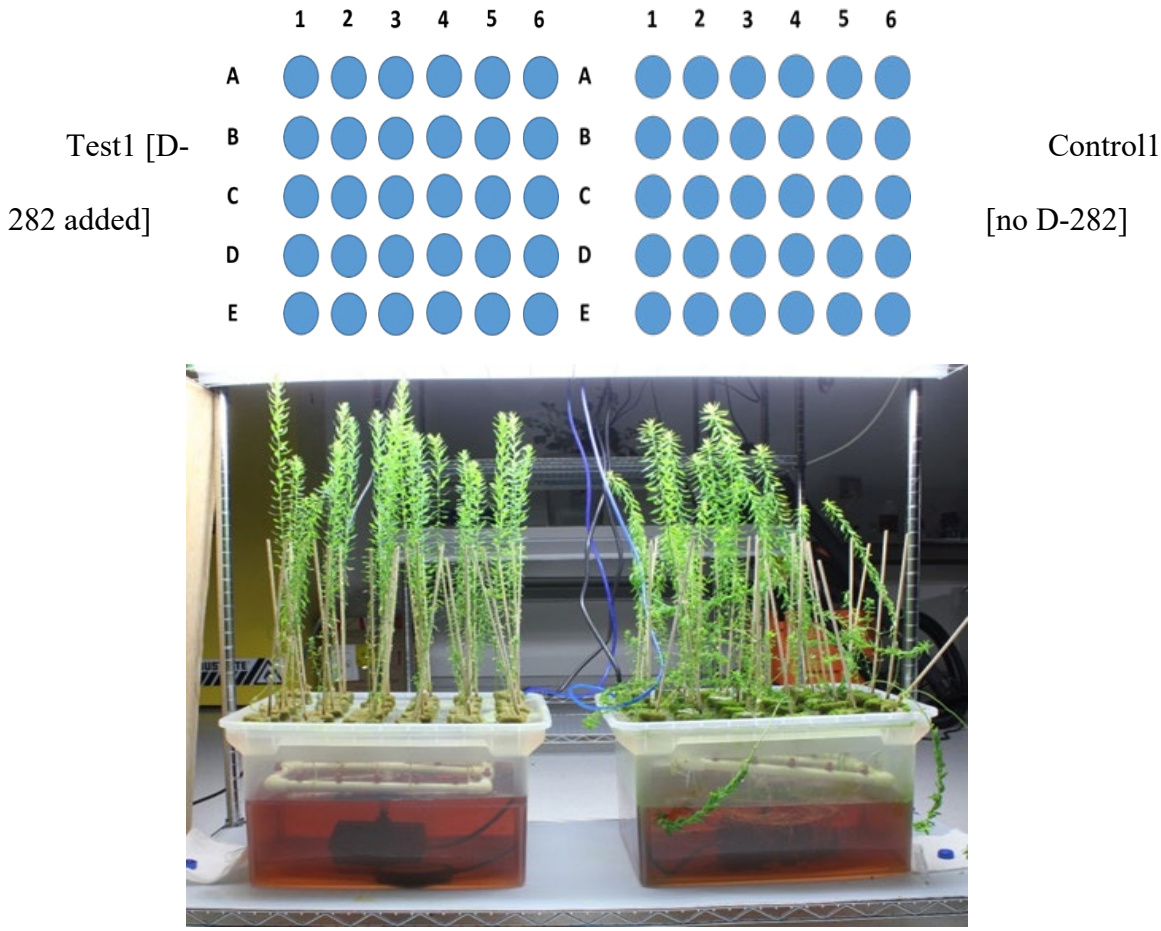
2.2 Materials and Methods:

For each trial, we utilized the following procedure. 1) Grow a population of *Linum usitatissimum* (golden flax) plants from seeds obtained from Whole Foods in 1.5” by 1.5” rock wool cubes (Grodan). 2) Place a 7x9 sheet of cubes in a sterilized flat black 10”x20” seed germination tray with holes. 3) Place the tray with holes into a corresponding sterilized tray without holes. 4) Place the seeds in the center well of each rock wool cube and tamp down to ensure proper seating. 5) Make an initial nutrient solution per the General Hydroponics Flora

Series-Simple Recirculating guidelines for seed germination, i.e., 2.5 ml FloraMicro, 2.5 ml FloraGro, and 2.5 ml FloraBloom per gallon of water. 6) Add the root growth promoter *Azospirillum brasilense* to the nutrient solution by adding 2 tablespoons of "Azos," a commercially available lyophilized root growth promoting powder purchased from Whole Foods. 7) Titrate the solution to a pH of 6.0 before applying it to the rock wool seed bed. 8) Add the solution until the seed bed is saturated, approximately half a gallon of nutrient solution. 9) Place a clear plastic dome over the seed tray and place the tray atop a seedling heat mat (Super Sprouter). 10) Place the tray under a 4' fluorescent grow light fixture (Portable Luminaire, Issue No. BM-18744) that contains four 8500K fluorescent bulbs and that provide the photoperiod of 16 hours on and 8 hours off. The heat mat increases the temperature approximately ten degrees over ambient (72ºF). 11) Allow the seedlings to grow for 18 days before transplanting them to custom made aeroponic growth vessels. We used clear storage containers from Home Depot and Plexi Glass cut to fit the tops of the storage containers with 1.5" holes to hold the plants. 12) Sterilize two vessels and prepare them for transplantation. 13) Make four gallons of early growth nutrient solution per the General Hydroponics Flora Series – Simple Recirculating guidelines, i.e., 7.5 ml FloraMicro, 10 ml FloraGro, and 2.5 ml FloraBloom per gallon of water, and titrate to pH 6.0. 14) Place 7 liters of the nutrient solution in each of the vessels. We dissolved 2.0 grams of Day Glo D-282 optical sensitizer powder in the nutrient solution of one of the vessels (test). 15) Outfit the vessels with 4" O_2 disks, connected to ECOPLUS – Eco Air 2 air pumps, and our custom nutrient spray systems, connected to ECOPLUS ECO-185 submersible pumps. 16) Tie 60 Germinated seedlings which exhibit roots extending from the wool to wooden stakes for support and record their heights. 17) Measure the heights from the top of the rock wool cube to the top of the plant with a tailor's tape measure. 18) Place the rock wool cubes in the Plexi

glass openings of the vessel top and place the vessels under the grow lights. 19) Connect the submersible pump and air pump to timers that cycle off and on at 15-minute intervals. 20) Connect the lights to a timer that is set to irradiate the system for 16 hours per day, from 05:00-21:00. We recorded the position of each plant by row and column, according to Figure 8.

Figure 8. Picture and Schematic Diagram of Placement of Staked Flax Plants in the Test and Control Aeroponic Systems.

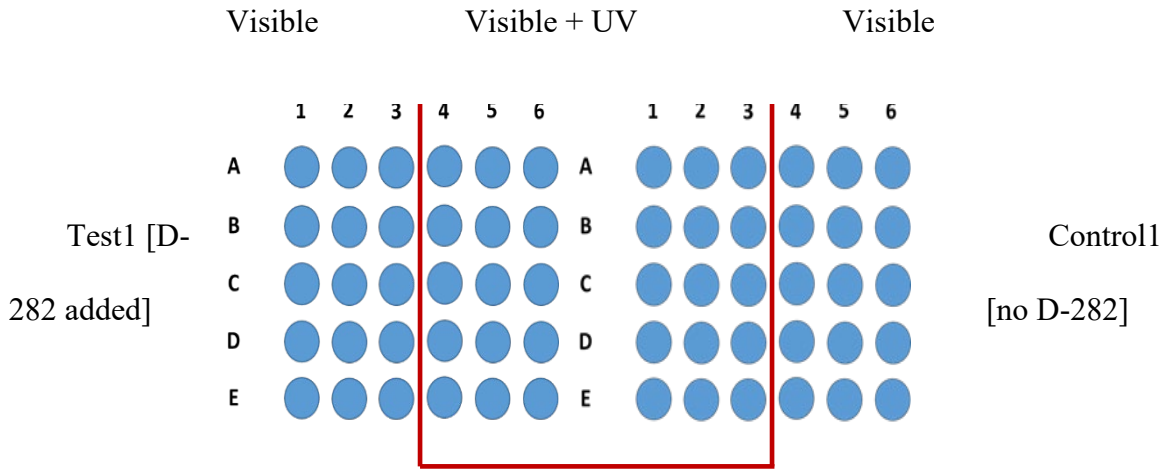


21) Measure the heights of both the control and test populations and record the heights for 40 days from the time of transplantation. Perform measurements of the surface area of the leaves by choosing a height, i.e., 12 inches, and measure the length and width of leaves at that height

across all plants. 22) Replace the nutrient solution weekly as recommended by the Flora Series guidelines.

On day 40, we modified the system through a reconfiguration of the light sources to selectively add UV radiation (UVP UVGL-25 Compact UV lamp 4 watt. P/N 95-0021-12) to half of each population as shown in Figure 9. We made the light barrier from cardboard.

Figure 9. Schematic Diagram of the Lighting Configuration for Phase 2.



Note. Phase 2 study on the effects of UV versus visible irradiation on the growth of flax, with and without D-282 dye.

We continued to measure and record the heights for an additional 14 days.

Upon completion of the growth phase of these experiments, we dried the plants for three days and then removed the rock wool. We weighed the dried plant matter to allow for a comparison of the total biomass of each population.

After we completed phases 1 and 2, we made a comparison between the growth rates of the Azos microbiome in media with and without the addition of D-282. For this phase of the

experiment, we used the following procedure. 1) Start a culture of additive by dissolving .05 g of Azos in 25 ml of Davis Minimal Broth (DMB) and allowing to grow overnight in an Incu-Shaker mini (Benchmark) incubator shaker at 37ºC and 130 revolutions per minute. 2) Inoculate three 25 ml flasks (Fischer Scientific) of DMB blank media and three 25 ml flasks of DMB media containing D-282 at a concentration of 0.3 g/l with Azos culture to an optical density reading of 0.1. 3) Using Nanodrop 2000c (Thermo Scientific), take optical density (OD) readings at 600nm. 4) Top the flasks with Silicone Sponge Closure Flask Stoppers (Thomas Scientific, part # 1203K23) and place the flasks in an Incu-Shaker mini (Benchmark) incubator shaker at 37ºC and 130 revolutions per minute. 5) Take OD readings every 30 minutes until a growth curve is obtained including the lag phase, exponential phase, and plateau phase. We repeated this experiment two more times for statistical relevance and to determine if there were any effects on the growth of the bacteria.

2.3 Data:

The flax plants grown with D-282 present in the nutrient solution exhibited blue fluorescence in the leaves under 254 nm UV illumination, as shown in Figure 10 a versus Figure 10 b for the control group, which contains no D-282.

Figure 10. Image of Flax Plants Under UV Illumination

Note. The plant grown with D282 (A) added to the media displays a strong blue fluorescence. The plant grown in the control population (B) exhibits a corresponding lack of strong fluorescence.

Data for the stem length, root length and overall biomass of the flax plants are shown in appendix A. Data for the microbiome optical density readings are shown in appendix B.

2.4 Results:

Phase 1:

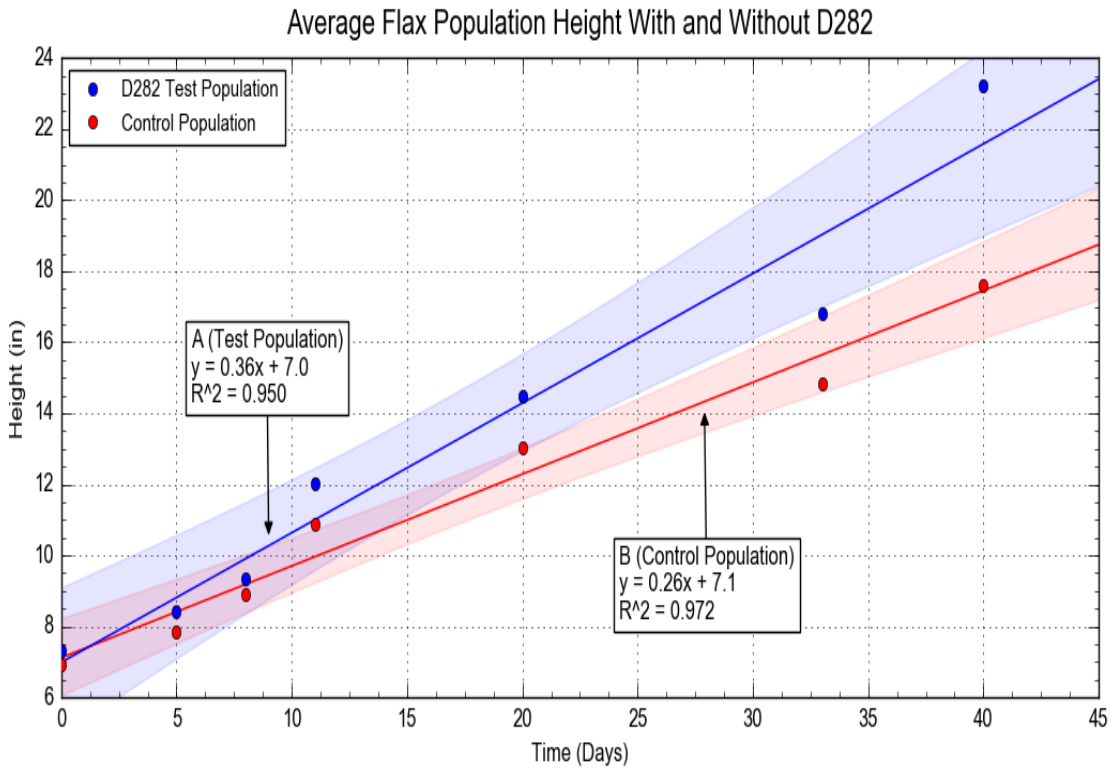
As shown in Figure 10, fluorescence can be seen in the leaves of the flax plants, which were exposed to D282 in the nutrient solution, under UV irradiation. This result demonstrates that the brightener can be introduced hydroponically and taken up by the flax. For this system,

the optical brightener travels through the root system, up the xylem, and finally localizes in the leaves. The optical brightener emits blue light, i.e., between 410 nm and 450 nm with a peak at 440 nm, when 254 nm UV light is incident on the leaves.

Based on the growth data, we plotted the overall average plant heights for the control and test populations and found linear growth trends for each population, as shown in Figure 11. The D-282 test population (A) exhibited a significantly higher stem growth rate, i.e., 0.36 ± inches/day, relative to that of the control group (B), i.e., 0.26 ± inches/day. Based on these results, we estimate that the stems of the test group grew 139% (0.36/0.26) faster than those of the control group under 8500K fluorescent lighting. This may be explained by the additional photosynthetically useful energy transformed by the fluorophore.

The following graphs summarize the overall growth behavior of the test and control flax plants, under visible fluorescent, i.e., 8500K, irradiation over a 40-day period.

Figure 11. Overall Growth Rates of Flax During Phase 1.



Note. Overall Growth Rates, i.e., inches/day, of flax plants (A) with and (B) without the D-282 additive under visible radiation. The bands correspond to a 95% confidence interval. Note: The test and control groups exhibit linear growth correlation coefficients, R^2, of 0.950 and 0.972, respectively.

<u>Phase II:</u>

With the addition of 254 nm UV light to the system, the linear growth rates of the crop portion of the plants with D282 showed an even greater increase than the crop portion of the plants with no D282. The control group exhibited growth of about 0.18 inches per day while the test group showed a growth rate of about 0.32 inches per day. Based on these results, the flax stems of the test group grow at a rate ~78% (0.36/0.18) faster than those in the control group,

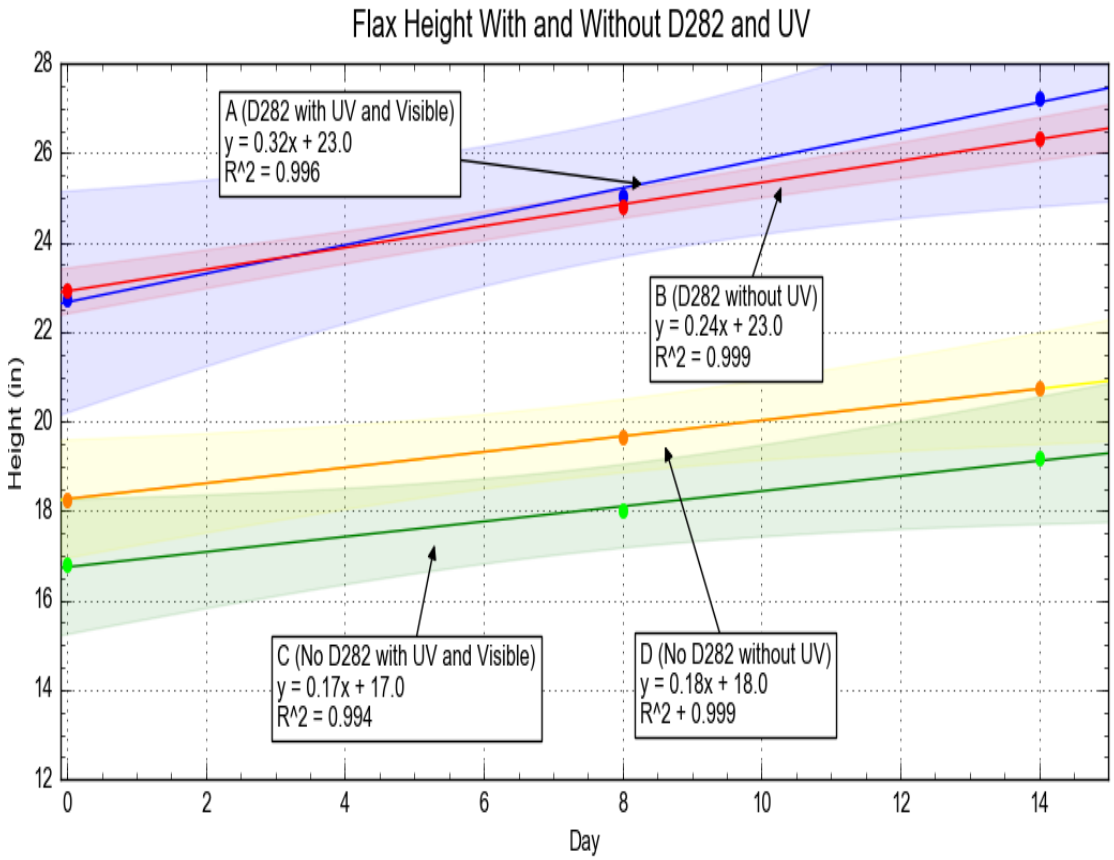
when exposed to fluorescent and 254 nm lighting, Again, this may be attributed to the increased flux of useful photosynthetic energy that is being transformed by the D-282 upon exposure to the added UV light.

While the addition of D282 caused a marked increase in plant stem growth, as can be seen above, it had no significant impact on leaf surface area. Specifically, the overall average surface area of the leaves in the test group, i.e., $0.32 \pm 0.1\ \text{in}^2$ (2σ), was statistically identical with that of the control group, i.e., $0.34 \pm 0.1\ \text{in}^2$ (2σ). However, the impact of D-282 on the length of the roots was significant and noteworthy. Specifically, the root length difference between the two groups was highly significant, and similar in magnitude to the differences between stem lengths. The control group expressed an average root length of 6.3 ± 2.0 (2σ) inches, whereas the test group had an average root length of 2.33 ± 0.51 (2σ) inches.

Overall, the above ground portion of the plant exhibited enhanced growth rates for the test versus control populations, while the root system in the test population appears to exhibit a proportionately smaller size compared to the control group. Considering the trends with the increase in stem length, the similarity in leaf size, and the decrease in root growth caused by the addition of D282, the overall biomass showed an interesting result. The control group showed a statistically identical overall biomass of 4.90 ± 0.87 grams compared to the test groups 4.92 ± 0.58 grams, which suggests that the addition of D282 to the system shifted plant growth processes preferentially from roots to stems.

The following graphs summarize the overall growth behavior of the two populations under visible and ultraviolet irradiation over a 14-day period.

Figure 12. Overall Growth Rates of Flax Plants for Phase 2

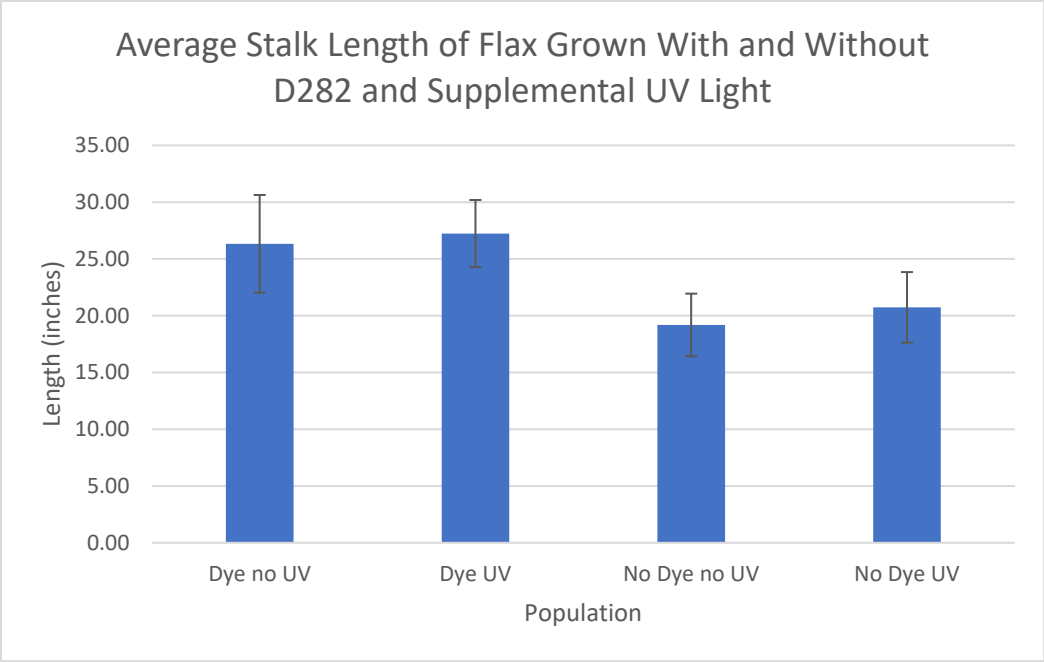


Note. Growth curves, i.e., inches/day, of Flax plants with and without D282 additive under visible light and visible + UV radiation, with the slopes corresponding to growth rates in inches per day. Starting points correspond to average plant height of each group at the end of phase one. The growth curves are defined as follows: (A) D-282 with UV that exhibits an R^2 of 0.996, (B) D-282 without UV that exhibits an R^2 of 0.999, (C) control with UV that exhibits an R^2 of 0.994, and (D) control without UV that exhibits an R^2 of 0.999.

The following graphs summarize the average stalk length (Figure 13), average leaf surface area (Figure 14), average root length (Figure 15), and average overall biomass (Figure

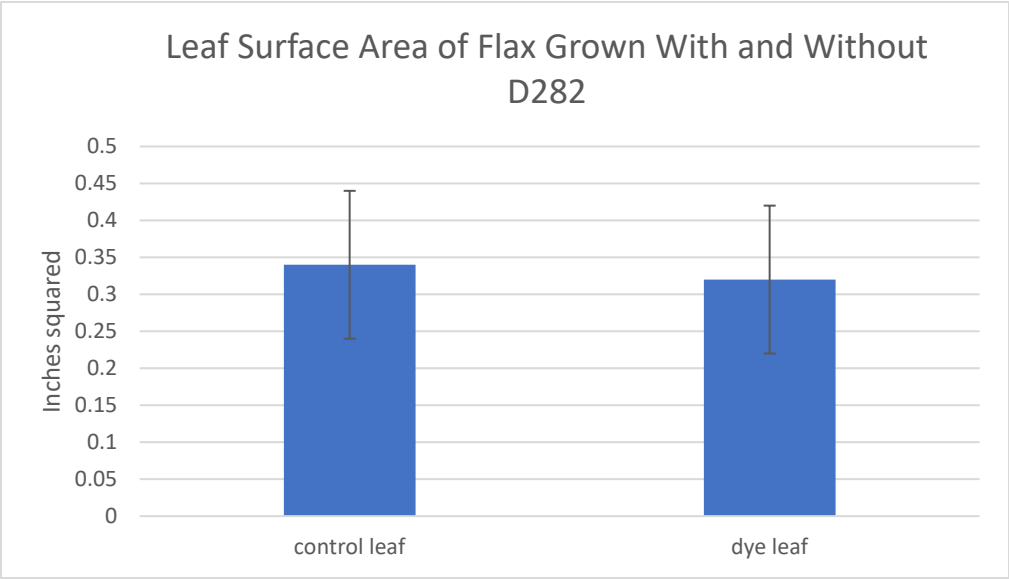
16) at the conclusion of phase 2. For Figure 13 N < 15 and for Figures 14-16 N < 30 due to the exclusion of plants with bent or broken stalks.

Figure 13. Average Stalk Length After Phase 2



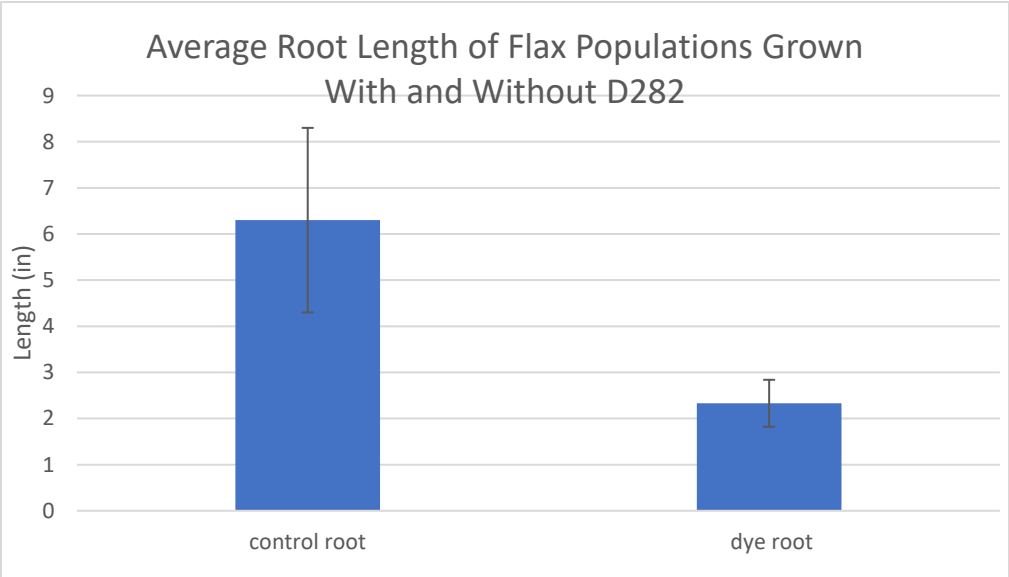
Note. Average stalk length of the test (Dye) and control (No dye) populations with and without the addition of supplemental UV light. N=11.

Figure 14. Average Leaf Surface Area After Phase 2



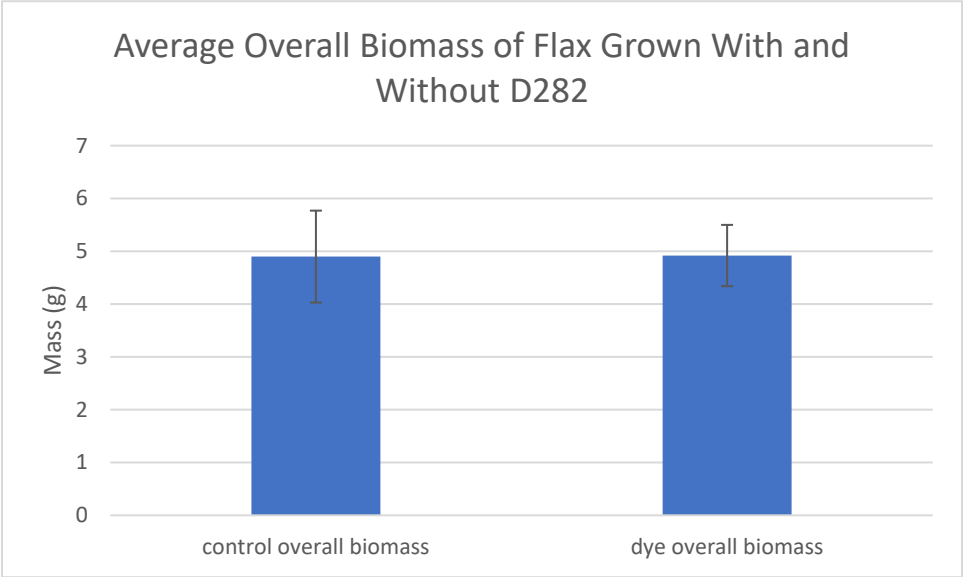
Note. N=22

Figure 15. Average Root Length After Phase 2



Note. N=22

Figure 16. Average Overall Biomass in Grams After Phase 2.

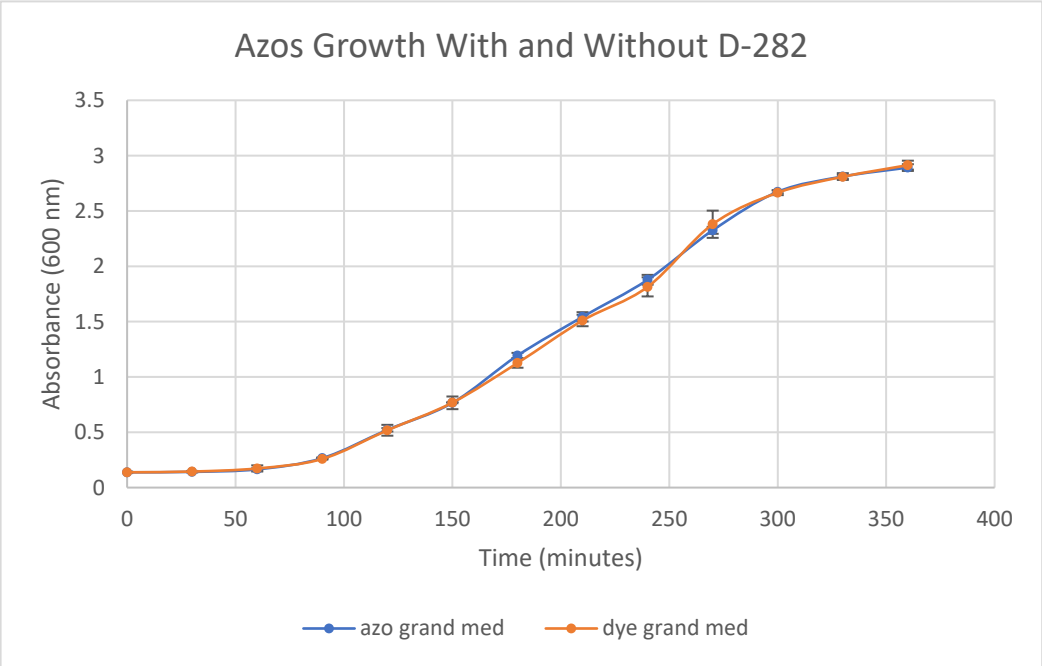


Note. N=22

<u>Phase 3:</u>

The following graph, shown in Figure 17, is a composite growth curve for the Azos microbiome grown in a nutrient media with and without D-282. Increased optical density readings at 600 nm light absorption correlates with an increase in bacterial concentration. These data show that the control and test populations grew at statistically identical rates, as the 2σ error bars between the control and test populations overlap at each point along the curve. Based on this result, we can assert that macroscopically the bacterial growth was not inhibited by the addition of D-282 at the concentration of optical brightener used in these studies.

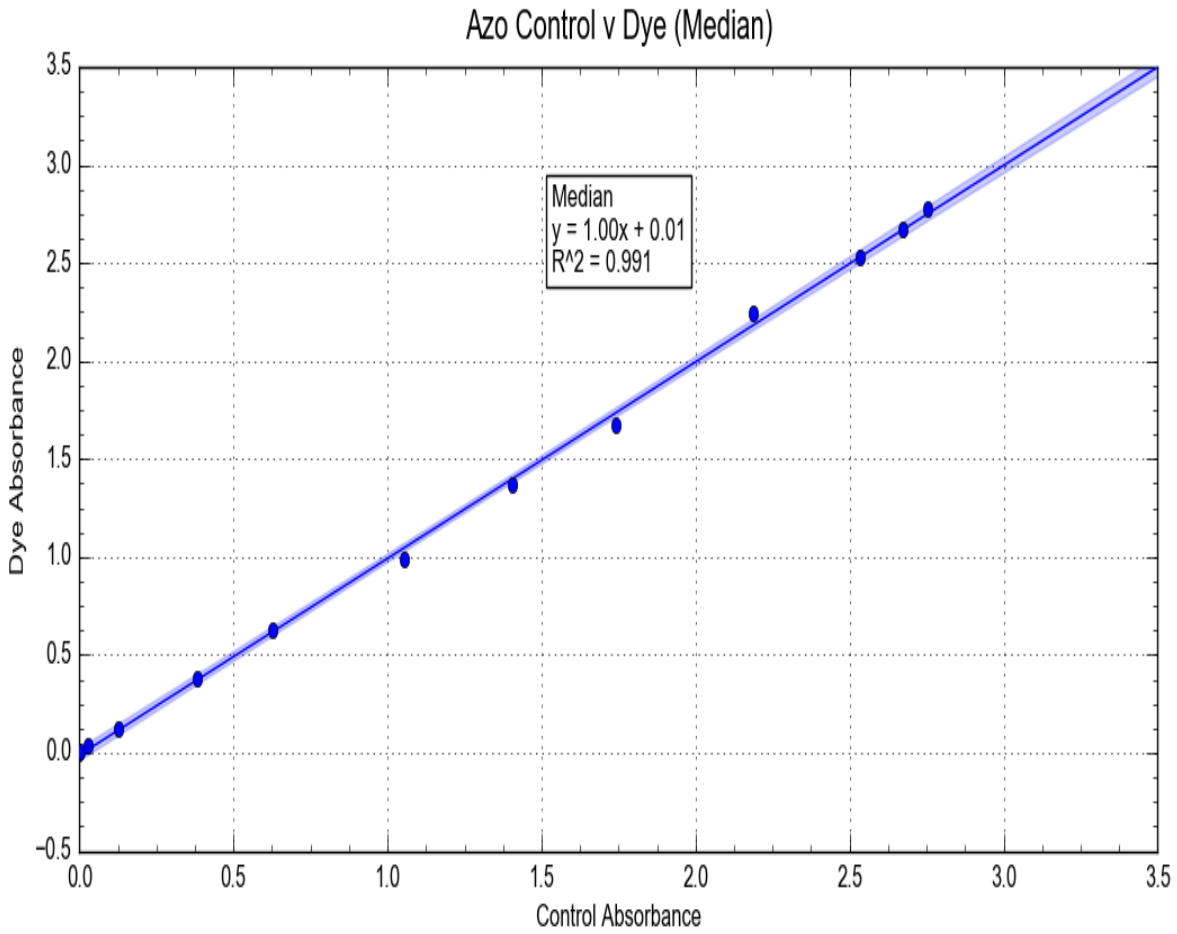
Figure 17. Composite Growth Curve of Supplied Microbiome.



Note. Grown in media a) with (red curve) and b) without (blue curve) D282. The initial Azos OD reading was 0.1 with a D282 concentration of 0.3g/l. N=9.

The graph shown in Figure 18 shows the corresponding median absorbances of the test group versus the absorbances of the control group at each sample time. This plot provides a more direct comparison, than Figure 17, of the temporal trends in optical density between the control and test microbiomes.

Figure 18. Absorbance of Control Versus Test Populations.



Note. The band denotes a 95% confidence interval with an R^2 of 0.991.

The comparison of optical density readings of the supplied microbiome imply that the samples grew at a statistically identical rate, as the slope of the plot is 1.00. It is noteworthy that all data points touch or are included within the 95% confidence interval, i.e., the shaded narrow blue region.

2.5 Discussion:

The hydroponic addition of D-282 to flax plants under various lighting conditions had a marked and significant effect on flax growth behavior. Specifically, the relative growth rates for the test versus control populations were as follows: stem growth rates increased 39% under visible illumination and 78% with supplemental UV light, leaf surface area was unaffected, and root lengths decreased 63%. Interestingly, the overall biomass growth rates were statistically identical. These results partially support prediction #1 that the addition of D-282 to hydroponically grown flax plants increases stem growth rates, but with a corresponding decrease in root growth rates. They also do not support prediction #2, as we observed no significant overall increase in plant biomass.

The flax absorbed the dye and distributed it throughout the plant tissue, especially to the leaves, i.e., the photosynthetically active portion of the plant. The dye, through fluorescent energy transfer, absorbed damaging UV light and emitted photosynthetically useful blue light that overlapped with the blue absorption bands of Chlorophyll. The photosynthetic reaction centers present in the leaves readily use blue light to trigger photosynthetic activity. The increase in available blue light led to enhanced stem growth rates, when compared to control populations, and a corresponding decrease in the plant root growth rates. This observed effect was possibly due to sequestering phosphorous, which is vital for root formation. (80) A possibly useful future study would consider the effects of D-282 on a plant population's roots if the root system was allowed to establish and mature before the application of the D-282.

Using the optical density method of measuring bacterial growth, it becomes apparent that the dye has no adverse macroscopic impact on the bacterial system's ability to proliferate, at the

dye concentrations used in these studies. The bacteria grown with D-282 showed a statistically identical growth curve as that grown without it. These results support our initial hypothesis that the dye, at the concentrations needed to show an increase in stem growth, would have little or no adverse macroscopic effect on the test microbiome. Another useful future study would examine the impact of the optical brightener on the microbiome's genome.

Table 1. Summary of Predictions and Results for Experiment 1.

Prediction	Result	
increase in plant crop growth rate	increased in length between 39%-78%	Supports prediction
increase in plant root growth rate	decreased in length by 63%	Doesn't support prediction
increase in plant leaf growth rate	showed no difference in leaf area	Doesn't support prediction
increase in overall biomass	showed no difference in overall biomass	Doesn't support prediction
no inhibition of microbiome growth rate	showed no difference in growth rate	Supports prediction

In summary, the hydroponic addition of D-282 fluorescent blue dye, as an optical brightener, to flax plants provides more photosynthetically useful light flux by converting UV light to blue light, which in turn yields significantly increased stem growth rates. However, it is interesting to note the observed tradeoff between an increase in stem growth rates and a balancing reduction in root growth rates. The D-282s impact to attenuate root growth may be mitigated by adding the D282 to the plants after the roots have fully matured. Also, the addition of D-282 appears to exhibit no statistical macroscopic impact on the system's microbiome growth rates.

CHAPTER III: ADDITION OF FLUOROPHORE TO CYANOBACTERIA

3.1 Effects of the Addition of a Fluorophore to Media on *Synechococcus elongatus*

In this section, we look at the addition of a fluorescent trans-stilbene optical brightener, Day-Glo D282, to the media of *Synechococcus elongatus*. As discussed previously, chapter 2 page 1, D282 shifts light energy from the non-photosynthetically useful portion of the spectrum, i.e., UV, to the photosynthetically useful region, i.e., blue (400 to 460 nm). We investigate the growth rates of *Synechococcus elongatus* in the presence and absence (control) of D282 through optical density readings as a function of growth time. We also investigate the minimum inhibitory concentration (MIC) of D282 to *Synechococcus elongatus*. This is the lowest concentration of a substance that exhibits a deleterious effect on the growth rate of an organism. Finally, we investigate the genomic effects on *Synechococcus elongatus* due to exposure to D282. Specifically, sequencing the genome after approximately 300 generations of growth in the presence of D282 to determine if any mutations occur.

We hypothesize that the addition of D282 to the growth media of *Synechococcus elongatus* will lead to an increase in the growth rate of the cyanobacteria and, therefore, an increase in the overall biomass generated. We will observe this by comparing optical density readings of a test and control population as OD corresponds to the amount of bacteria present in a sample. This effect would be due to the additional photosynthetically useful light supplied to the system by D282 shifting light from the unused UV (254 nm) portion of the spectrum to the blue (400-460 nm). Also, we hypothesize that at a high enough concentration of D282 we will find an inhibitory or toxic effect as is common with many additives that may be introduced to a growing bacterial sample. Finally, we hypothesize that the D282, at concentrations sufficient to

see an increase in growth rate but well below the MIC, will not induce genomic changes to *Synechococcus elongatus*. We think this will be the case because its toxicity to other algae (*Scenedesmus subspicatus*) has been reported at a much higher concentration, acute toxicity at 1000 mg/l and chronic toxicity at 500 mg/l, than used in this study and generally low toxicity amongst other organisms. (81).

3.2 Materials and Methods:

Phase 1:

We procured an ancestral cell line of *Synechococcus elongatus*, UTEX 2973, from the UTEX Culture Collection of Algae at the University of Texas at Austin along with their premade growth media, BG-11, for use in this study. We conducted all growth experiments in BG-11 media. We grew all cultures in an Incu-shaker Mini incubator shaker (Benchmark) at 27°C and 80 rpm under a fluorescent grow light fixture (Portable Luminaire grow lights, issue No. BM-18744) that contained four 8500K fluorescent bulbs. The photonic flux measured as 150 $\mu mol/m^2/s$ at the sample locations. We utilized the following procedure to prepare for these experiments. 1) Grow the ancestral strain to saturation, 5 days, in 10 mL of BG-11 then serial dilute out to 100000x with BG-11 and plate on BG-11 agar. 2) Select one unique colony and grow it up over 5 days in 10 mL of BG-11 to confluency and use a 1 mL aliquot, in phase 2, to perform a minimum inhibitory concentration (MIC) assay to determine the sub-lethal concentration of D-282 (fluorescent dye, Day-Glo) to be utilized for selection purposes (0.1 g/L). 3) Create a stock solution by inoculating 99 ml of BG-11 media with 1 ml of the sample from UTEX in a 250 ml beaker (Fischer Scientific). 4) Place the culture in the incubator shaker at 27°C and 80 rpm under a fluorescent grow light fixture that contained four 8500K fluorescent bulbs. 6) Grow the culture for 5 days until the media

becomes saturated and use this stock culture to inoculate the 25 ml flasks (Fischer Scientific) for this experiment. 7) Pellet the remainder of the stock culture with an AccuSpin Micro 17R centrifuge (Fischer Scientific) and store at -80ºC for future use.

We then utilized the following procedures. 1) Inoculate 15 sterilized 25 ml flasks (5 control and 10 treatment) containing 9.9 ml of BG-11 media with 0.1 ml of stock culture and top the flasks with silicone sponge closure flask stoppers (Thomas Scientific, part #1203K23). 2) Grow the treatment group in media containing a 0.1 g/L concentration of D-282. 3) Place the flasks in the incubator shaker at 27°C and 80 rpm under the same grow light fixture as described above. We marked the placement of the flasks in the incubator with white lab tape and rotated the flasks clockwise, every 8 to 12 hours, through the positions throughout the course of the experiment. 4) Measure the photonic flux at each position with a photometer (Quantum Flux photometer, Apogee Instruments) to ensure each position receives an equal amount of flux, i.e., 150 $\mu mol/m^2/s$ of photons.

Figure 19. Scanning Electron Micrograph of S. elongatus.

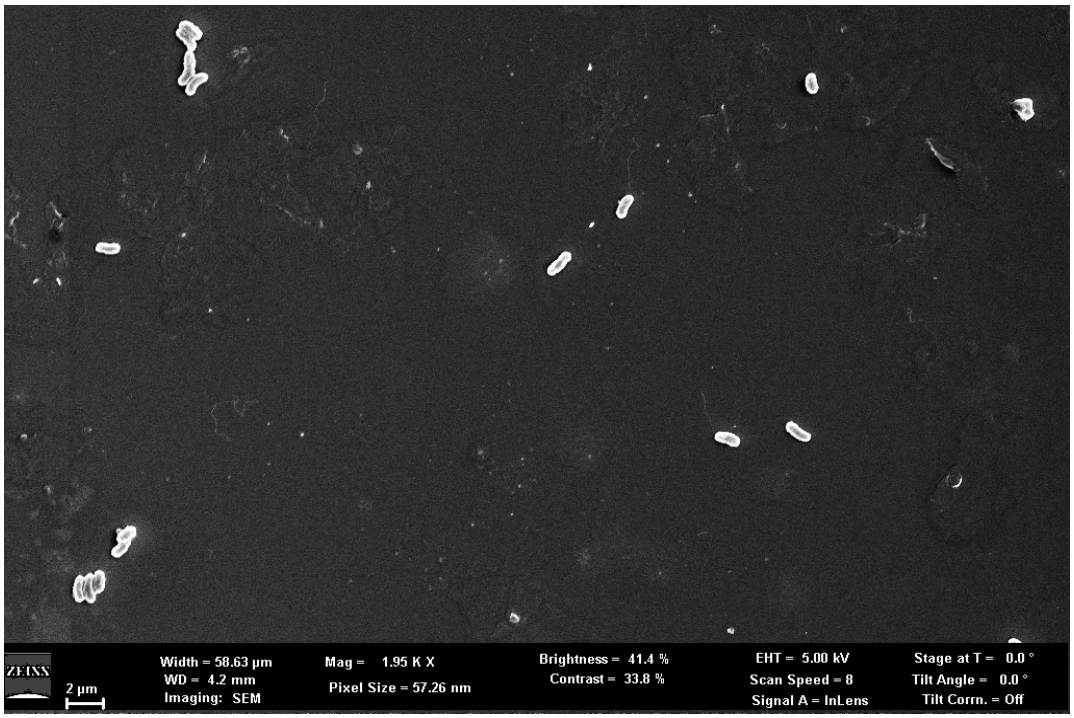


Figure 20. S. elongatus Cultures in Incubator-shaker.

Note. With (right) and without (left) D282.

5) Monitor the *S. elongatus* growth over a 7-day span using optical density measurements taken with a NanoDrop 2000c (Thermo Scientific). 6) Take measurements at 0 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, 168 hours, and 192 hours. We took the optical density readings at 750 nm to ensure no interference from absorption of light by photosynthetic pigments within the cells.

Phase 2:

To establish MIC, we utilized the following procedure. 1) Grow *Synechococcus elongatus* cultures in a 96-well plate with a gradient of D282 concentrations, i.e., 0, 60, 120, 250, 500, 750, 1000, 1750, 2500, and 5000 mg/l. 2) Seal the plates with paraffin and keep them in the incubator

shaker between measurements. 3) Measure optical density over a 5-day span with a 96-well plate reader and use the measurements to identify the lowest concentration that would inhibit the growth.

Phase 3:

For the genomic study we utilized the following procedure. We made 8 treatment flasks and 4 control flasks from the stock culture. 1) Inoculate sterilized flasks containing 9.9 ml of BG-11 media with 0.1 ml of stock culture. The treatment group was grown in media containing a 0.1 g/L concentration of D-282. 2) Place the flasks in the incubator shaker under the light fixture. 3) Grow the cultures for ~7 days to allow the media to saturate. 4) Make a fresh flask for each sample and serially transfer samples by taking a 0.1 ml aliquot from each culture and adding to 9.9 ml of BG-11 media with (treatment) and without (control) D282. 5) After the first week, pellet the cultures and store at -80ºC until ready to extract DNA for early-stage genomic sequencing. 6) Repeat step 4 for 15 weeks. 7) After 15 weeks, pellet the samples for late-stage genomic sequencing. 8) From both early and late-stage samples, extract DNA using the EZNA Bacterial DNA extraction kit (Omega Bio-tek®, Cat No. D3350-02) as per manufacturer instructions. We determined DNA concentrations fluorometrically using the QuantiFluor® ONE dsDNA System (Promega Corporation, Madison, WI, USA) on a Quantus® fluorometer (Promega Corporation). We performed measurements according to the manufacturer's recommendations.

9) Prepare genomic libraries using the standard protocol as described in the manufacturer's instructions (Illumina, Nextera DNA Flex Library Prep Reference Guide). All the reagents used are included in the Nextera DNA Flex kit (Illumina, cat. Nos. 20,018,704, 20,018,705). The depth of coverage, the average number of reads that cover the whole genome, of the sequencing runs ranged from 20x to 80x, with most exceeding 40x coverage. An overview of the workflow is

depicted below in figure 21. 10) For each sample, load 200 ng of DNA into a stripped PCR tube with nuclease-free water added to bring the volume up to a total volume of 30 µl. 11) Add 20 µl of well-mixed tagmentation master mix to bring the final volume up to 50 µl. 12) Seal the stripped PCR tubes and incubate at 55°C for 15 min. 13) After the tagmentation reaction, perform post tagmentation cleanup by adding10 µl of Tagment Stop Buffer to each reaction and resuspend the beads. 14) Seal the tubes and incubate at 37°C for 15 min in a thermal cycler, and then place on a 96-well plate magnet (Thermo Fisher Scientific, cat. no. AM10027) for 3 min (or until the solution is clear). 15) Meticulously discard the supernatant and then perform two rounds of washes with each round involving the following steps: remove the plate from the magnet, add 100 µl of Tagment Wash Buffer, place the tubes back on the plate magnet for 3 min (or until clear), and then discard the supernatant. 16) Resuspend the washed beads in 100 µl of Tagment Wash Buffer before placing the tubes on the magnet for a further 3 min (or until clear).

17) Perform amplification of the tagmented DNA utilizing a cycle PCR reaction. In each stripped PCR tube, make 40 µl of PCR master mix by mixing 20 µl of Enhanced PCR Mix with 20 µl of nuclease-free water. Thereafter, completely remove the Tagment Wash Buffer from each sample well prior to removal of the plate from the magnet. 18) Add 40 µl of PCR master mix to each sample. 19) Add 10 µl of index adapters, according to the index kit configuration being used for 96 plex [dual index], and 10 µl of primer mix and mix the samples by pipetting 4 times. 20) Seal the tubes and place them in a thermal cycler with a heated lid, and run them using the following PCR parameters: 68°C for 3 min, 98°C for 3 min, then 5 cycles of 45 seconds at 98°C, 30 seconds at 62°C, and 2 min at 68°C, before a final minute at 68°C. 21) Centrifuge the PCR products for 1 min at 280 x g.

22) Carry out cleanup with the aid of a double-sided bead purification procedure, using SPRI beads (termed Sample Purification Beads, SPBs). 23) Transfer the samples to new PCR tubes and place them on a plate magnet for 5 min. 24) Transfer 45 µl of the clear supernatant to fresh stripped PCR tubes. For each sample, prepare a diluted solution of SPBs by mixing 45 µl of SPBs with 40 µl of nuclease-free water. 25) Add 85 µl of the solution to each sample in a fresh tube containing the 45 µl of supernatant and mix the samples by pipetting 4 times. 26) Incubate the tubes at room temperature for 5 min and place them on a plate magnet for a further 5 min (or until clear). 27) During incubation, add 15 µl of the undiluted SPBs to fresh tubes. 28) For each sample, transfer 125 µl of supernatant from the first set of tubes to the second set of fresh tubes containing 15 µl of undiluted SPBs and mix the sample by pipetting 4 times. 29) Incubate the tubes at room temperature for 5 min then place them back on a plate magnet for 5 min. 30) Discard the supernatant and add 200 µl of 80% ethanol to each well of the plate on the magnet followed by a 30 second incubation. 31) Remove the ethanol and wash the beads again with 80% ethanol before allowing the tubes to air dry on the plate magnet for 5 min to ensure complete removal of ethanol. 32) Remove the tubes from the magnet and add 32 µl of Resuspension Buffer to the beads. 33) Resuspend the beads and incubate at room temperature for 2 min. 34) Place the tubes on the plate magnet for 2 min before transferring 30 µl of the supernatant of each sample containing the DNA library to a fresh set of stripped tubes for pooling.

For pooling, which is the last step before sequencing, 35) add the libraries by volume (5 µl per sample) into a 1.5 mL tube prior to sequencing. 36) Verify the quality of the final library using D1000 Screen Tape (Agilent Technologies, CA, USA) following the manufacturer's instruction. 37) Measure the library concentration using fluorometric quantification with the dsDNA binding dye (as earlier described) and dilute to 12pM with Resuspension Buffer. 38) Run the pooled

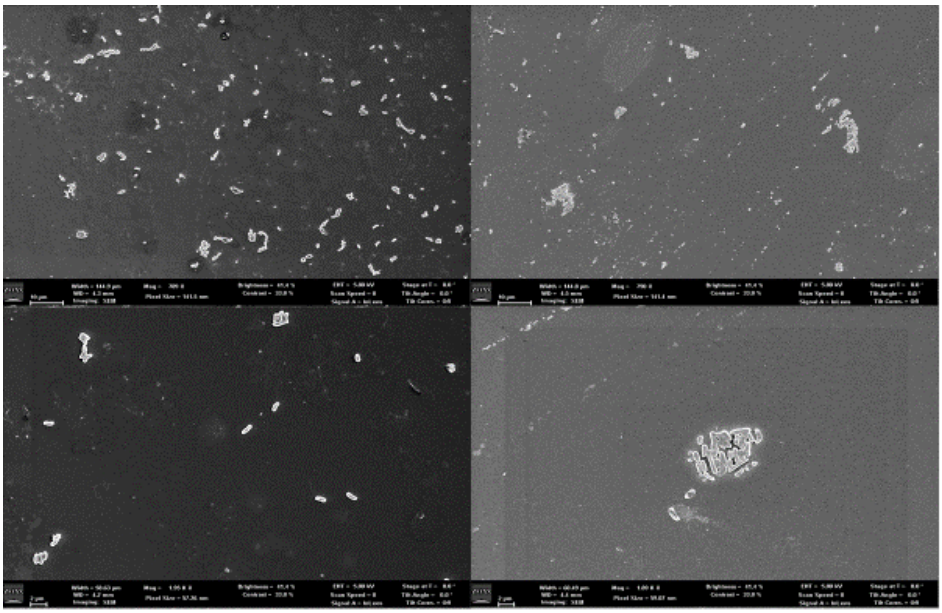
libraries on an Illumina MiSeq (Illumina, San Diego, CA) using the MiSeq v3 reagent kit. Sequence alignment and variant calling from the samples was achieved by use of the breseq 0.30.0 pipeline set to polymorphism mode (-p) and default parameters (83). The pipeline uses three types of evidence to predict mutations, read alignments (RA), missing coverage (MC), and new junctions (JC) (84), and any reads that indicate a difference between the sample and the reference genome that cannot be resolved to describe precise genetic changes are listed as "unassigned." These unassigned reads are not described nor interpreted here.

Figure 21. Nextera DNA Flex Library Prep Workflow Overview.

3.3 Data:

We observed the morphology and aggregation of the *Synechococcus elongatus* samples grown with and without D282 utilizing scanning electron microscopy (SEM).

Figure 22. Scanning Electron Micrograph of Control (no D282) and Test (D282).



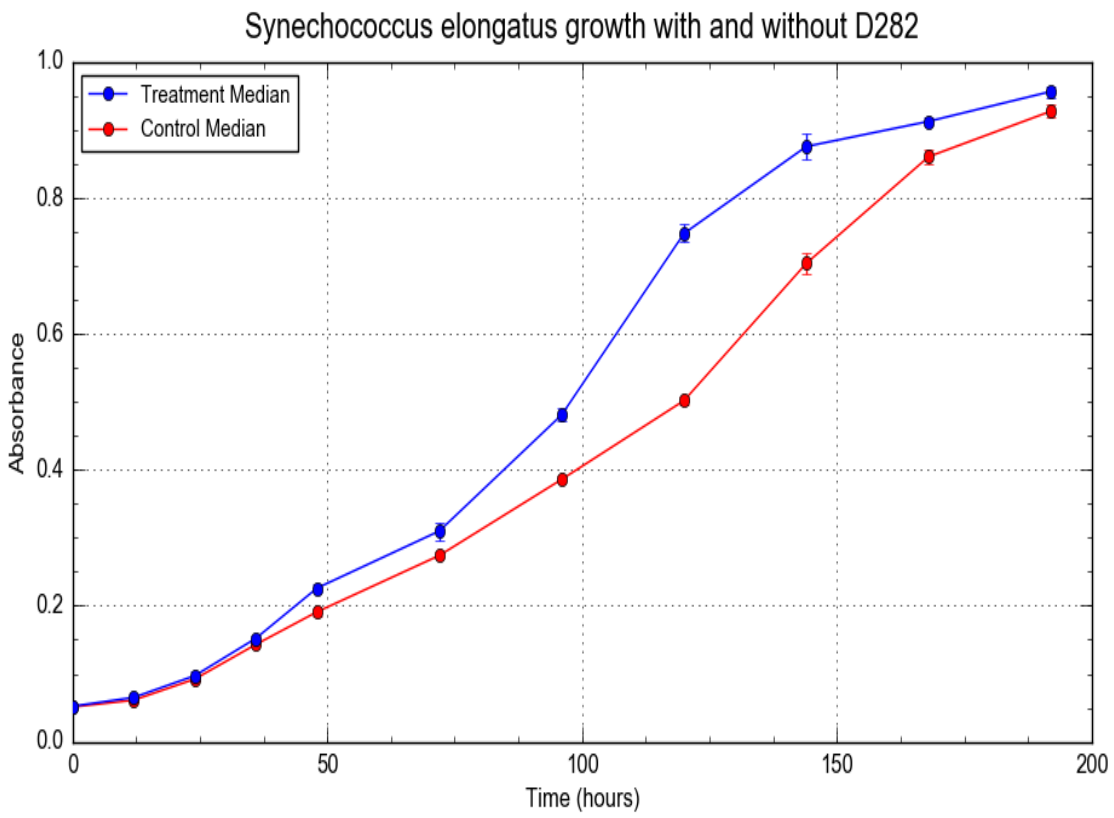
Control D282 Treatment

Raw data for optical density growth curves, MIC optical density readings and genomic sequencing results are shown in appendices C, D, and E.

3.4 Results:

The following graph summarizes the overall growth behavior of the test and control *Synechococcus elongatus*, under visible fluorescent, i.e., 8500K, irradiation over a 7-day period

Figure 23. Optical Density Growth Curves of Synechococcus elongatus.

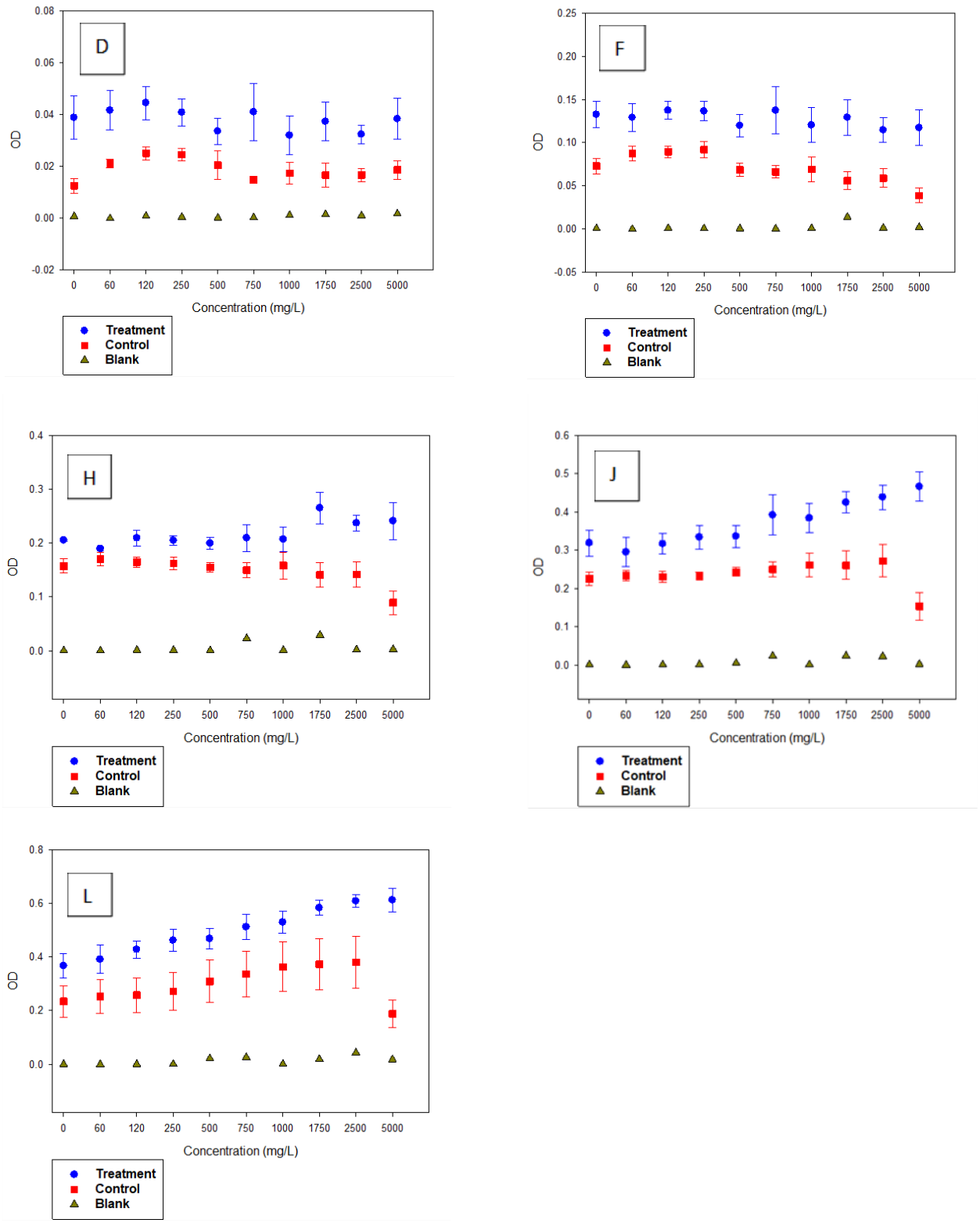


Note. With (blue) and without (red) D282 additive under visible radiation.

Based on the above graph the D282 population grew at a markedly faster rate than the control group. This increased growth may be explained by the additional photosynthetic activation energy supplied by the fluorophore.

The following graphs summarize the MIC optical density readings over the course of 5 days for the control and treatment groups.

Figure 24. Optical Density MIC Readings.



Note. Measurements taken at D) 24, F) 48, H) 72, J) 96, and L) 120 hours.

After an initial lag phase, i.e., 36 hours, until hour 72 the control group showed a slight inhibition around 1 g/l where the treatment group did not. The control group appears to acclimate slightly to the concentrations up to 2.5 g/l through the end of the readings, but at 5 g/l it is always inhibitory. For the treatment group, the inhibitory effect is only seen at the 5 g/l concentration. This is most likely due to these samples being grown in the presence of D282 prior to the MIC experiment and developing a slight resistance to any inhibitory effect.

Based on the genomic data shown in table 2 below, we see that there is no mutagenic effect of D282 on *S. elongatus* at the concentrations we are using to increase growth rates. The only mutations that we found are four sweeps, those occurring in 100% of reads across a treatment group, and four adaptation mutations, those occurring in 10 to 90 percent of reads across a treatment group. The sweeps occurred in genes Synpcc7942_0095, Synpcc7942_0361, Synpcc7942_0918, and Synpcc7942_1475. Two adaptations occurred in the genes Synpcc7942_0095 and Synpcc7942_2577. The hard sweeps, with 100% coverage, are assumed to be ancestral as they are present at 100% in both treatment and control samples. Since we found the adaptation mutations to be present, in different percentages, in the treatment and control populations, we do not believe D282 caused these mutations. However, they may be ancestral mutations, or polymorphisms that occurred before the experiment. The variance in the frequency between control and test populations may be due to the D282 having a mitigating or enhancing effect on the adaptation. We can report detecting variance in the genomics at day 60 but the function relative to D282 is unknown.

Table 2. Genomic Changes in *S. elongatus* Grown in Presence of D282.

Position	Mutation	SeT1	SeT2	SeT3	SeT4	SeT5	SeT6	SeT7	SeT8	SeC1	SeC2	SeC3	SeC4	Annotation	Gene	Description
889,961	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.193	0.000	0.000	L322L (CTC→CTA)	*Synpcc7942_0884* ←	translation elongation factor 1A (EF 1A/EF Tu)
1,906,685	C→A	0.000	0.000	0.000	0.000	0.244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	V50V (GTG→GTT)	*Synpcc7942_1839* ←	conserved hypothetical protein
1,919,783	T→A	0.000	0.000	0.000	0.140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	G140G (GGT→GGA)	*Synpcc7942_1851* →	Ferredoxin nitrite reductase
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	Two-component transcriptional regulator, winged helix family.
93,060	C→A	0.370	0.265	0.195	0.000	0.319	0.417	0.341	0.249	0.690	0.702	0.637	0.592	G94C (GGC→TGC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	0.670	0.595	0.749	1.000	1.000	0.672	0.684	0.731	0.280	0.203	0.354	0.208	V18F (GTC→TTC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	conserved hypothetical protein
487,631	G→A	0.000	0.000	0.000	0.000	0.122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	S247F (TCC→TTC)	*Synpcc7942_0499* ←	hydroxyneurosporene O methyltransferase
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	long chain fatty acid CoA ligase
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	sodium dependent bicarbonate transporter
1,572,744	G→T	0.000	0.000	0.000	0.126	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	A87S (GCT→TCT)	*Synpcc7942_1520* →	SSU ribosomal protein S20P
1,579,838	C→G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.201	0.000	0.000	R249G (CGG→GGG)	*Synpcc7942_1524* →	DNA directed RNA polymerase
1,786,217	T→A	0.116	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	D480V (GAT→GTT)	*Synpcc7942_1716* ←	diguanylate cyclase/phosphodiesterase with PAS/PAC sensor(s)
2,561,810	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.113	0.000	L13F (TTG→TTT)	*Synpcc7942_2480* ←	Prolyl 4 hydroxylase, alpha subunit
1,644,608	A→G	0.000	0.000	0.000	0.161	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (172/ 534)	*Synpcc7942_1580* ← / → *Synpcc7942_1581*	hypothetical protein/Peptidase M14, carboxypeptidase A
2,662,566	Δ150 bp	0.182	0.209	0.255	0.000	0.000	0.182	0.344	0.203	0.560	0.630	0.639	0.544	coding (378 527/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein
2,662,727	Δ7 bp	0.294	0.385	0.267	0.565	0.462	0.407	0.405	0.623	0.610	0.600	0.500	0.400	coding (360 366/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein

3.5 Discussion:

The objective of this section was to determine the effects of the addition of a fluorophore to media on *Synechococcus elongatus*. *S. elongatus* is a unicellular, obligate photoautotroph that represents a preeminent model for studying photosynthesis (82). In this study, we explored the effect of the fluorescent trans-stilbene optical brightener, Day-Glo D282 on the growth of *S. elongatus* and also examined genomic changes associated with the growth of *S. elongatus*. We found that the *S. elongatus* samples grown with D282 grew markedly faster than the samples grown without D282. This result supports our hypothesis that the shifting of UV light energy, which is not photosynthetically useful, to blue light, which is photosynthetically useful, by D282 would lead to an increase in the growth and biomass of *S. elongatus*.

We also found that at high enough concentrations, i.e., initially 1 g/l, the D282 has an inhibitory effect on the growth of *S. elongatus*. The concentration where this effect occurs is well above the concentration needed to increase the growth rate, i.e., 0.1g/l, which also supports our initial hypothesis.

Finally, we found that there were no genomic changes caused by the D282 at concentrations that caused an increased growth rate in *S. elongatus*. This result supports our hypothesis that, due to the reportedly low toxicity of D282 at concentrations sufficient to see an increase in growth rate but well below the MIC, we would not induce genomic changes to *Synechococcus elongatus*.

Table 3. Summary of Prediction and Results for Experiment 2.

Prediction	Result	
increase in cyanobacteria growth rate	Test grew faster than control	Supports prediction
inhibitory effect on growth rate at high concentration	MIC found at high concentrations	Supports prediction
no mutagenic effect at experimental concentration	no significant mutations	Supports prediction

CHAPTER IV: EFFECTS OF NARROW BAND LIGHT ON CYANOBACTERIA

4.1 Study of Effects of Narrow Band Light Environments on *Synechococcus elongatus*

In this section, we look at the effects of narrow band light environments on the cyanobacteria *Synechococcus elongatus*. As light travels deeper into water, the longer (red) wavelengths of light are absorbed or scattered, and the shorter (blue) wavelengths penetrate deeper due to their higher energy. Consequently, fewer and fewer wavelengths are available for use by photosynthetic organisms the deeper they are and the more they rely on accessory pigments. To mimic this effect, we studied the growth of *S. elongatus* in broad spectrum white (control) light, narrow band orange light, and narrow band green light. We investigated the effects of the light environments on the genomics of *S. elongatus* as well as the pigments Chlorophyll-a, beta-carotene, and phycocyanin. We chose these pigments for their prevalence within *S. elongatus* as well as their absorption spectra. We also targeted various genes of the light selected samples that are responsible for the production of these pigments and analyze their expression through qualitative real time polymerase chain reaction (qrtPCR). The targeted genes enable the biosynthetic pathways of various pigments. For example, phytoene synthase (crtB) enables beta-carotene production. For phycocyanin, chlorophyll-a, and a housekeeping gene we targeted phycocyanin subunit beta (cpcB), chlorophyll synthase (chlG), and phosphoenolpyruvate carboxylase (ppC) respectively. Finally, we investigate the concentration of the targeted pigments in the light selected samples to determine any effect the narrow band environment has on pigment production.

We hypothesize that the growth of *S. elongatus* in narrow band light environments will induce significant genomic changes that alter the concentration of pigments to optimize photosynthesis. Also, we hypothesize that since each pigment absorbs slightly different

wavelengths of visible light, limiting the light to specific wavelengths will lead to an increase in concentration of pigments that absorb those specific wavelengths. Specifically, 1) growth in green light will lead to an increase in beta-carotene production and 2) growth in orange light will lead to an increase in phycocyanin production. Finally, we hypothesize that the expression of genes in the biosynthetic pathway for the production of targeted pigments will be altered. In particular, 1) growth in green light will lead to increased expression of crtB and 2) growth in orange light will lead to an increase in cpcB.

4.2 Materials and Methods:

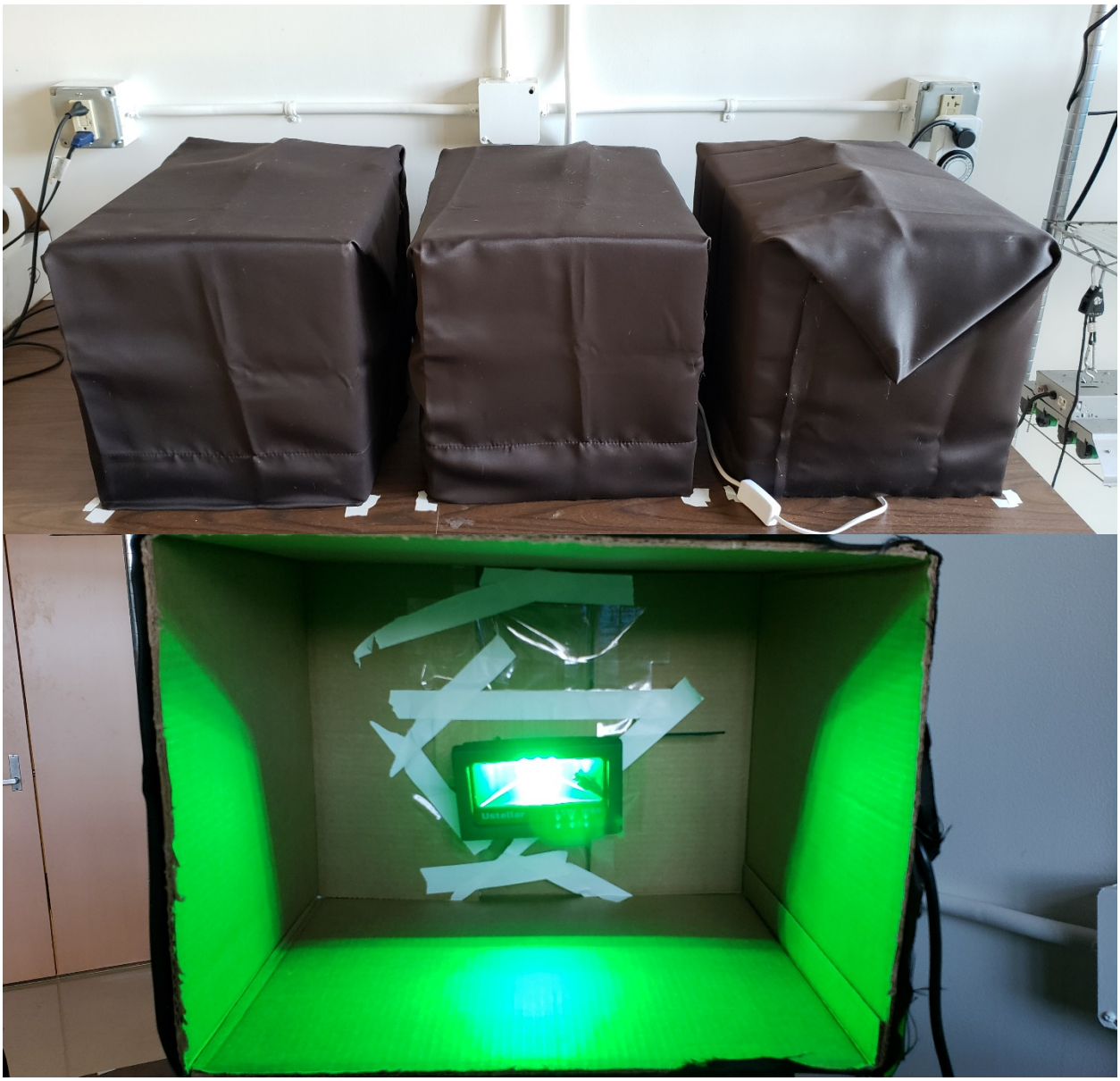
Phase 0 Growth in Narrowband Light Environments

We used an ancestral line of *S. elongatus*, UTEX 2973, from the UTEX Culture Collection of Algae at the University of Texas at Austin along with their premade growth media, BG-11, in this study. We utilized the following procedure to create a stock solution. First, inoculate 99 ml of BG-11 media with 1 ml of the sample from UTEX in a 250 ml flask (Fischer Scientific). The culture was placed in a Benchmark Incu-shaker Mini incubator shaker at 27°C and 80 rpm under a Portable Luminaire (Issue No. BM-18744) fluorescent grow light fixture that contained four 8500K fluorescent bulbs. The culture grew for 7 days until the media was saturated and was used to inoculate the sterilized flasks for this experiment. 8 25 ml flask (Fischer Scientific) were inoculated from the stock culture for each treatment and topped with silicone sponge closure flask stoppers (Thomas Scientific, part # 1203K23).

To begin the experiment, we utilized the following procedure. 1) Inoculate sterilized flasks containing 9.9 ml of BG-11 media with 0.1 ml of stock culture. 2) Place the flasks in custom made growth chambers, cardboard boxes covered in blackout curtain material, purchased from Home

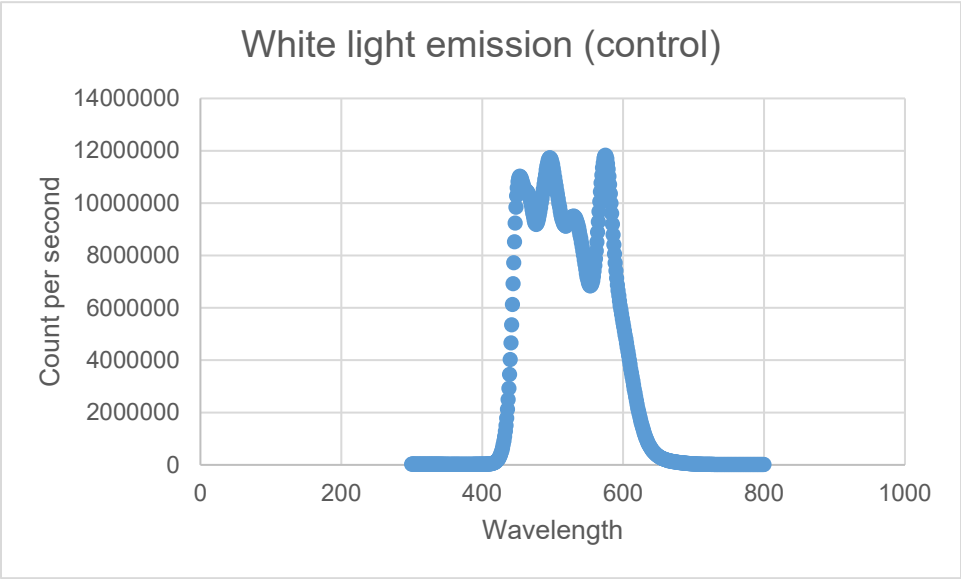
Depot, fitted with adjustable LED lights (Ustellar 15W RGB LED Flood lights) that allowed us to supply only specific narrowband wavelengths of light to the samples.

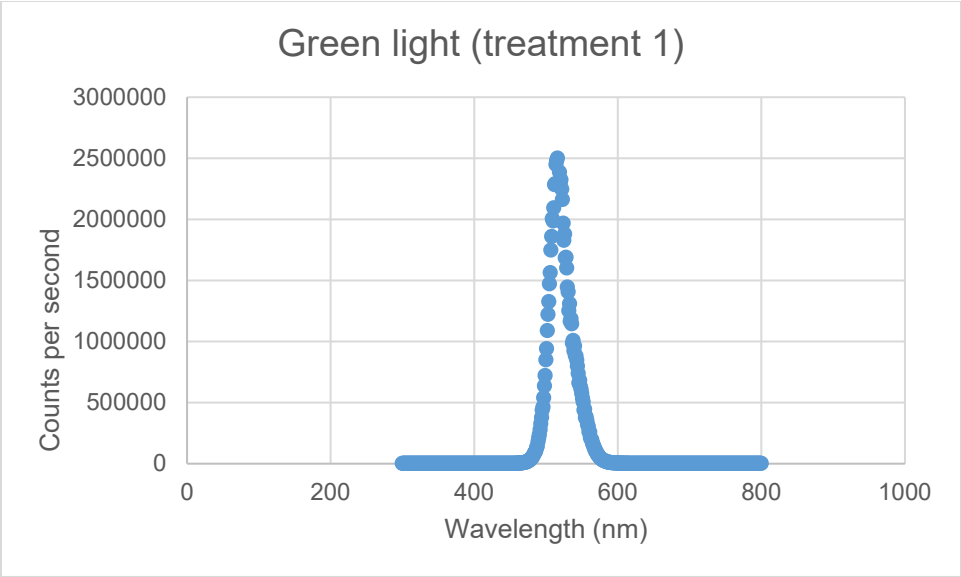
Figure 25. Images of the Exterior and Interior of Custom-Made Grow Chambers with Adjustable Wavelength LED Lights.

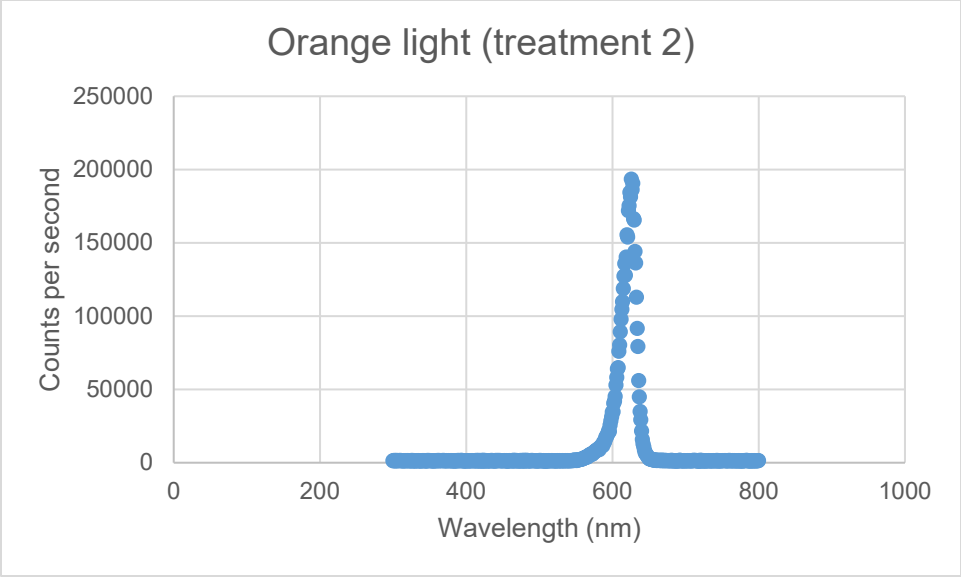


3) Grow the treatment groups in green (500-525 nm) and orange (610-630 nm) narrow band light while growing the control in broad spectrum white light. For each light type, we measured the emission spectra with a UV/Vis Spectrofluorometer (Horiba Scientific, Jobin Yvon Technology). The spectra are shown in Figure 26.

Figure 26. Emission Spectra of Lights Used in Sample Growth Chambers.

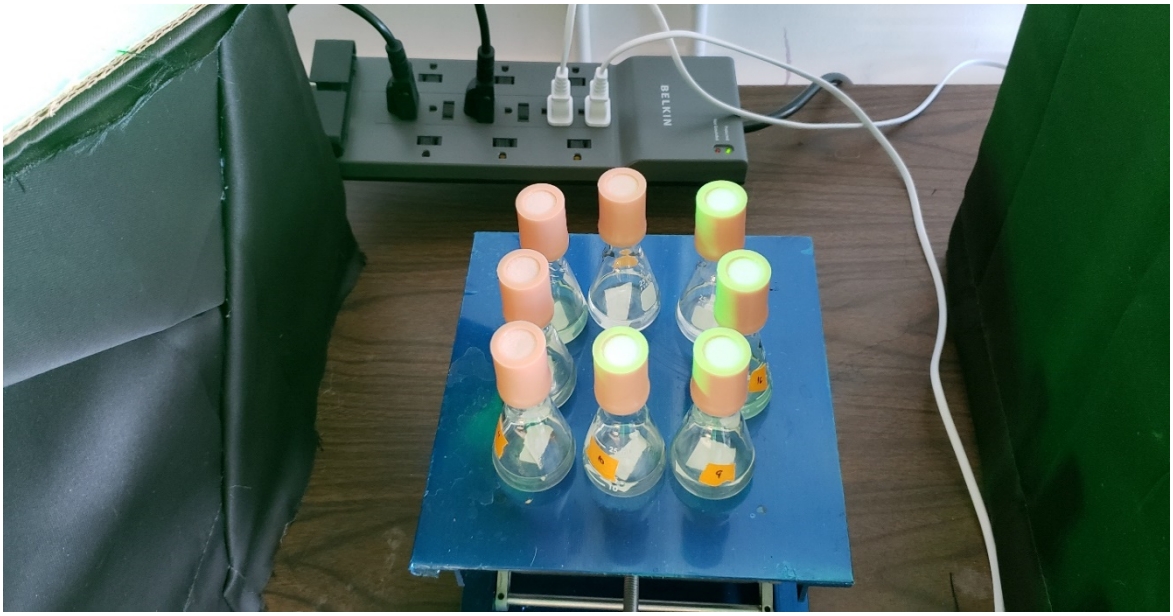






We placed the samples on lab jacks to bring them closer to the light source and marked positions with white lab tape to ensure that each sample received an equal amount of photonic flux across all samples and all light sources. We used a Quantum Flux photometer (Apogee Instruments) to measure the photonic flux, i.e., 100 µmol/m^2/s of photons. 4) Grow the samples at room temperature, i.e., 23°C, with periodic shaking to prevent shading and biofilm formation.

Figure 27. Placement of Flasks Within the Grow Chambers.



Note. White tape marks indicate where flux is equal. Each position in each chamber received equal flux.

Phase 1. Evolution and Whole Genome Sequencing

5) Allow the samples to saturate the media over the course of ~5 days. 6) At this point, inoculate fresh flasks and return to the grow chambers. This process was repeated for 120 days to provide an early and late set of samples for genomic analysis. 7) After the first growth cycle, i.e., 5 days, extract DNA from the samples and sequence it for the early-stage genomic analysis. 8) On day 120, extract DNA from the samples and sequence it for the later stage genomic results. 9) Perform DNA extraction with an EZNA Bacterial DNA extraction kit (Omegabiotek, Cat No. D3350-02) as described in Chapter 3. 10) Perform DNA library preparation and indexing according to the Nextera DNA library prep kit and Nextera DNA CD index kit guidelines, as

described in Chapter 3. 11) Perform sequencing with a MiSeq reagent kit version 3 600 cycle whole genome sequencing cartridge for the Illumina Miseq, as described in Chapter 3.

Phase 2. Pigment Biosynthetic Pathway Gene Expression using Quantitative Real Time Polymerase Chain Reaction (qrtPCR)

We utilized the following procedure to analyze gene expression for our selected genes from the pigment biosynthetic pathway. 1) In the late stages of the experiment, extract and purify RNA using the RNeasy Mini Kit (Cat. No 74104, Qiagen Sciences), following the manufacturer's specifications for bacterial cells. 2) Quantify the total RNA using the NanoDrop 1000TM (Thermo Scientific, USA). 3) Conduct real time qPCR using a 7500 Fast Real Time PCR System (Applied Biosystem). 4) Perform the reactions with a Luna Universal One-Step RT-qPCR Kit (NEB# E3005L, New England BioLabs) according to the manufacturer's instructions. We designed oligonucleotide primers to target our chosen genes using Integrated DNA Technologies (IDT) design tools and purchased the Sybergreen tagged PrimeTime STD DNA primers from IDT as well. The primers used for expression were: For beta carotene, where we targeted phytoene synthase (crtB), the primers used consisted of the forward sequence, CGA TGT TGC CTT GGT GGA TA, and reverse sequence, ACG GTA GCA GTA GGT GTA GAG. For phycocyanin, where we targeted phycocyanin subunit beta (cpcB), the primers used consisted of the forward sequence, GGT TGC AGA AGG CAA CAA AC, and the reverse sequence, GCC AGG AGC AAT CAG AGA AG. For chlorophyll-a, where we targeted chlorophyll synthase (chlG), the primers used consisted of the forward sequence, CGG CTC GTT TGT CTC CTA TAT C, and the reverse sequence, CAA GGC AGA GCG ATG TAA CT. Finally, for the housekeeping gene, where we targeted phosphoenolpyruvate carboxylase (ppC), the primers used consisted of the forward sequence, AGT TCG CTT CAG GTG ATT CC, and the reverse sequence, GTG TAG AAG GGC

AGC TCA AA. 5) Use the SYBR scan mode on the real-time instrument. We performed triplicate reactions for each sample. The PCR programs were set as 1 cycle of reverse transcription at 55°C for 10 min; 1 cycle of initial denaturation at 95°C for 1 min; 40 cycles of denaturation at 95°C for 10 s followed by extension at 60°C for 30 s for each cycle. We set the melt curve for 60°C.

Phase 3 Ultra-Performance Liquid Chromatography (UPLC) for pigment concentrations

To measure concentrations of pigments, we used the following procedure. 1) Pellet the samples with an AccuSpin Micro 17R centrifuge (Fischer Scientific) at 10k rpm for 2 minutes and extract the pigments. 2) Perform a 2x 200 µl acetone extraction of the samples to obtain the chlorophyll and beta carotene. 3) Resuspend the remaining pellet in 1 ml of ethanol in a sealable tube and place in a hot oil bath at 120°C for 30 minutes to extract the phycocyanin. 4) Collect data with a UPLC-HRESIMS via a Q-Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA). An Ascentis Express ES-CN cyano column (Supelco, 50 mm x 2.1mm, 2.7 µm) with a flow rate of 0.3 mL/min and a column temperature of 35 °C was used. Starting conditions: 85% A, optima grade water with 0.1% formic acid, and 15% B, optima grade acetonitrile with 0.1% formic acid. We ran a gradient from 85:15 to 0:100 over 8 min, followed by an isocratic hold for 2 min at 0:100 with re-equilibration of the column at 85:15 for 2 min before the next injection. A 3 µL injection was used for all samples.

Phase 4 Morphology characterization

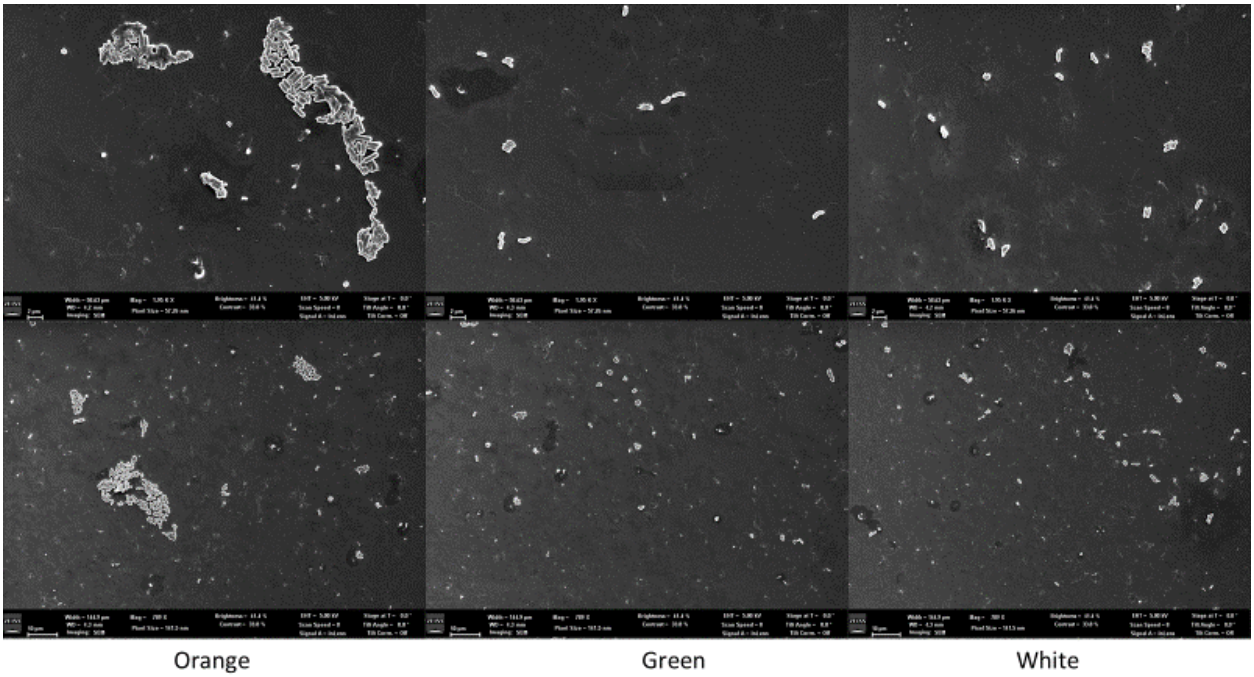
We utilized the following procedure to characterize morphological changes. 1) Drop-cast samples onto plastic coverslips by adding 50 µl of the culture to the coverslip. 2) Incubate the samples for 10 minutes at room temperature before removal of the excess media and culture from the coverslip. 3) Add enough Karnovsky's fixative to cover the surface and left them sit overnight

at 4ºC. 4) Remove the fixative and wash the samples 3 times with deionized water. 5) Perform an ethanol dehydration series (35%, 50%, 75%, 90%, 95%, 100% two times) each for ten minutes. 6) After the ethanol dehydration series, let the samples air dry. 7) Affix the samples to SEM stubs using carbon tape and sputter coat the samples with gold-palladium to a thickness of 5 nm using a Leica EM ACE200 sputter coater. 8) Image the samples using the Zeiss Auriga Scanning Electron Microscope (SEM) to look for aggregatory or morphological changes.

4.3 Data

The morphology and aggregation of the *S. elongatus* samples grown in the green treatment was observed to be the same as that grown in white light utilizing scanning electron microscopy (SEM). However, the samples grown in the orange treatment tended to aggregate more and were, on average, longer than that grown in white light. This can be seen in Figure 28.

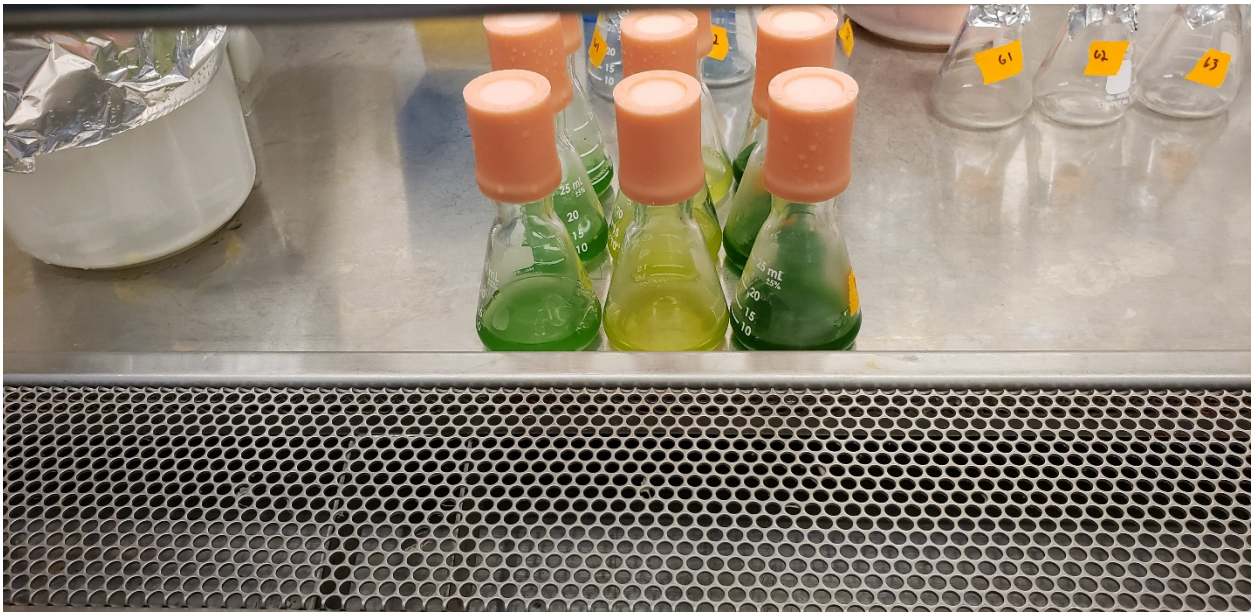
Figure 28. SEM Images of S. elongatus Grown in Various Light Treatments.



Note. The top row has a scale bar of 2 µm and the bottom row has a scale bar of 10 µm.

The color of the samples varied markedly from treatment to treatment as seen in Figure 29.

Figure 29. Color Variation in Light Selected Samples



Note. From left to right, grown in white light, orange light, and green light upon saturation.

The genomic data can be seen in Figure 30 below. The raw genomic data for early and late-stage samples can be found in appendices F and G respectively.

Figure 30. Raw Genomic Data for Early and Late-Stage Selection.

Position	Mutation	SynLE1	SynLE2	SynLE3	SynLE4	SynLE5	SynLE6	SynLE7	SynLE8	SynLE9	SynLE10	Annotation	Gene	Description
78,835	C→A	0.000	0.000	0.000	0.000	0.000	0.253	0.000	0.000	0.000	0.000	V13F (GTC→TTC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
92,978	T→C	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,060	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.137	0.150	G94C (GGC→TGC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.821	0.725	V18F (GTC→TTC)	*Synpcc7942_1183* →	conserved hypothetical protein
118,714	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.160	0.000	intergenic (+287/+21)	*Synpcc7942_0118* → / ← *Synpcc7942_0119*	aspartyl/glutamyl tRNA(Asn/Gln) amidotransferase subunit B/hypothetical protein
194,792	C→A	0.000	0.000	0.000	0.000	0.000	0.190	0.000	0.000	0.000	0.000	R242L (CGC→CTC)	*Synpcc7942_2139* →	probable glutathione S transferase
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_2192* →	diguanylate cyclase (GGDEF domain)
463,267	G→T	0.000	0.000	0.000	0.363	0.000	0.000	0.000	0.000	0.000	0.000	S161R (AGC→AGA)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
538,970	C→G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.129	D84H (GAT→CAT)	*Synpcc7942_0556* ←	two component transcriptional regulator, winged helix family
677,413	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.161	A158S (GCA→TCA)	*Synpcc7942_0683* →	potassium channel protein
688,215	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.114	intergenic (159/+119)	*Synpcc7942_0694* ← / ← *Synpcc7942_0695*	SSU ribosomal protein S1P/Protein of unknown function DUF193
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
1,033,221	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	intergenic (+9/ 79)	*Synpcc7942_1018* → / → *Synpcc7942_1019*	conserved hypothetical protein/4 alpha glucanotransferase
1,213,856	T→C	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	L126S (TTG→TCG)	*Synpcc7942_1415* ←	Proton translocating NADH quinone oxidoreductase, chain N
1,341,107	Δ1 bp	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.148	coding (474/1800 nt)	*Synpcc7942_1313* →	aspartyl tRNA synthetase
1,448,009	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133	0.000	L687L (CTC→CTA)	*Synpcc7942_1398* ←	Cellulose synthase (UDP forming)
1,466,670	C→A	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.000	G476C (GGT→TGT)	*Synpcc7942_1475* ←	sodium dependent bicarbonate transporter
1,526,404	C→T	1.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
1,587,695	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.182	0.000	0.000	0.000	E485* (GAA→TAA)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
1,965,769	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.094	G254G (GGA→GGG)	*Synpcc7942_1893* ←	ATPase
1,965,769	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.094	S267S (AGT→AGC)	*Synpcc7942_1892* →	Rhodanese like
2,070,588	G→T	0.000	0.000	0.211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	G193G (GGC→GGA)	*Synpcc7942_0361* →	conserved hypothetical protein
2,079,723	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.176	0.000	0.000	0.000	A140S (GCC→TCC)	*Synpcc7942_0918* →	long chain fatty acid CoA ligase
2,170,353	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133	T171M (ACG→ATG)	*Synpcc7942_2090* →	homoserine dehydrogenase
2,195,277	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.130	F151L (TTC→CTC)	*Synpcc7942_2114* →	histidine kinase
2,223,689	G→A	0.000	0.000	0.000	0.000	0.252	0.000	0.000	0.000	0.000	0.000	V30I (GTT→ATT)	*Synpcc7942_1475* ←	sodium dependent bicarbonate transporter
2,270,482	G→T	0.000	0.000	0.000	0.191	0.000	0.000	0.000	0.000	0.000	0.000	D397Y (GAT→TAT)	*Synpcc7942_1527* →	nitrogen assimilation regulatory protein
2,416,560	G→T	0.000	0.000	0.000	0.000	0.000	0.222	0.000	0.000	0.000	0.000	E58D (GAG→GAT)	*Synpcc7942_2010* ←	cytochrome c550
2,504,162	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	S598R (AGC→AGA)	*Synpcc7942_2431* ←	conserved hypothetical protein
2,596,739	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.128	E74K (GAG→AAG)	*Synpcc7942_2516* ←	hypothetical protein
2,662,727	Δ7 bp	1.000	0.000	1.000	1.000	0.833	1.000	1.000	1.000	0.857	0.714	coding (360 366/924 nt)	*Synpcc7942_0918* →	long chain fatty acid CoA ligase

Position	Mutation	SynLE_11	SynLE_12	SynLE_13	SynLE_14	SynLE_15	SynLE_16	SynLE_17	SynLE_18	Annotation	Gene	Description
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,060	C→A	0.168	0.278	0.000	0.323	0.364	0.000	0.192	0.179	G94C (GGC→TGC)	*Synpcc7942_0095* ←	Glucokinase regulatory like protein
93,288	C→A	0.759	0.742	0.603	0.636	0.552	1.000	0.797	0.760	V18F (GTC→TTC)	*Synpcc7942_0095* ←	conserved hypothetical protein
166,402	G→T	0.000	0.181	0.000	0.000	0.000	0.000	0.000	0.000	R307L (CGC→CTC)	*Synpcc7942_0166* →	diguanylate cyclase/phosphodiesterase with PAS/PAC and GAF sensor(s)/transcriptional regulator, MarR family
269,799	G→T	0.000	0.000	0.000	0.252	0.000	0.000	0.000	0.000	G48V (GGA→GTA)	*Synpcc7942_0275* →	sodium dependent bicarbonate transporter
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	conserved hypothetical protein
444,444	G→T	0.000	0.000	0.000	0.155	0.000	0.000	0.000	0.000	L148L (CTG→CTT)	*Synpcc7942_0455* →	Glucokinase regulatory like protein
447,878	A→G	0.000	0.105	0.000	0.000	0.000	0.000	0.000	0.000	F97L (TTC→CTC)	*Synpcc7942_0459* ←	two component transcriptional regulator, winged helix family
481,560	C→A	0.000	0.000	0.000	0.000	0.000	0.286	0.000	0.000	I198I (ATC→ATA)	*Synpcc7942_0493* →	conserved hypothetical protein
494,266	A→G	0.000	0.000	0.000	0.105	0.000	0.000	0.000	0.000	K177K (AAA→AAG)	*Synpcc7942_0507* →	two component transcriptional regulator, winged helix family
537,283	T→A	0.000	0.000	0.000	0.145	0.000	0.000	0.000	0.000	N230K (AAT→AAA)	*Synpcc7942_0554* →	conserved hypothetical protein
643,728	G→T	0.000	0.000	0.159	0.000	0.000	0.000	0.000	0.000	A80D (GCT→GAT)	*Synpcc7942_0649* ←	queuine tRNA ribosyltransferase
722,372	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.051	0.000	R650R (CGG→CGA)	*Synpcc7942_0727* →	ribosome recycling factor
793,466	G→T	0.000	0.000	0.000	0.000	0.235	0.000	0.000	0.000	R309S (CGT→AGT)	*Synpcc7942_0799* ←	long chain fatty acid CoA ligase
848,508	C→A	0.000	0.000	0.087	0.000	0.000	0.000	0.000	0.000	intergenic (+250/ 362)	*Synpcc7942_0851* → / → *Synpcc7942_0853*	nitrate transport ATP binding subunits C and D
860,868	G→C	0.154	0.000	0.000	0.000	0.000	0.000	0.000	0.000	A251P (GCT→CCT)	*Synpcc7942_0859* →	stage II sporulation protein D like
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	Glucokinase regulatory like protein
1,127,640	C→A	0.285	0.000	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (+113/+224)	*Synpcc7942_1110* → / ← *Synpcc7942_1111*	two component transcriptional regulator, winged helix family
1,192,766	G→T	0.000	0.000	0.122	0.000	0.000	0.000	0.000	0.000	intergenic (318/+26)	*Synpcc7942_1158* ← / ← *Synpcc7942_1159*	two component transcriptional regulator, winged helix family
1,202,173	G→T	0.000	0.191	0.000	0.000	0.000	0.000	0.000	0.000	P565T (CCT→ACT)	*Synpcc7942_1169* ←	Elongator protein 3
1,259,833	G→A	0.000	0.000	0.000	0.166	0.000	0.000	0.000	0.000	L375L (CTC→CTT)	*Synpcc7942_1237* ←	pyridine nucleotide transhydrogenase beta subunit
1,342,945	G→A	0.000	0.000	0.156	0.000	0.000	0.000	0.000	0.000	A487A (GCC→GCT)	*Synpcc7942_1314* ←	long chain fatty acid CoA ligase
1,393,137	C→A	0.160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	D1212Y (GAC→TAC)	*Synpcc7942_1357* ←	Glucokinase regulatory like protein
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	two component transcriptional regulator, winged helix family
1,585,991	G→T	0.000	0.000	0.148	0.000	0.000	0.000	0.000	0.000	E235D (GAG→GAT)	*Synpcc7942_1526* →	conserved hypothetical protein
1,680,129	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.000	V87V (GTG→GTT)	*Synpcc7942_1610* ←	tRNA i(6)A37 thiotransferase enzyme MiaB
1,710,882	Δ3 bp	0.000	0.130	0.000	0.000	0.000	0.000	0.000	0.000	coding (530 532/1521 nt)	*Synpcc7942_1643* →	conserved hypothetical protein
1,882,490	T→C	0.000	0.000	0.126	0.000	0.000	0.000	0.000	0.000	S504P (TCA→CCA)	*Synpcc7942_1811* →	long chain fatty acid CoA ligase
1,992,607	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	W80* (TGG→TAG)	*Synpcc7942_1917* →	sodium dependent bicarbonate transporter
1,998,975	T→A	0.000	0.000	0.134	0.000	0.000	0.000	0.000	0.000	Q61L (CAG→CTG)	*Synpcc7942_1922* ←	two component transcriptional regulator, winged helix family
2,081,946	G→A	0.000	0.000	0.000	0.145	0.000	0.000	0.000	0.000	W147* (TGG→TGA)	*Synpcc7942_2012* →	two component transcriptional regulator, winged helix family
2,164,639	G→T	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	intergenic (+43/+12)	*Synpcc7942_2083* → / ← *Synpcc7942_2084*	two component transcriptional regulator, winged helix family
2,442,754	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.052	0.000	R436R (CGC→CGA)	*Synpcc7942_2374* →	conserved hypothetical protein
2,648,802	G→T	0.159	0.000	0.000	0.000	0.000	0.000	0.000	0.000	R7S (CGC→AGC)	*Synpcc7942_2569* ←	long chain fatty acid CoA ligase
2,662,566	Δ150 bp	0.000	0.641	0.651	0.638	0.709	0.000	0.416	0.256	coding (378 527/924 nt)	*Synpcc7942_2577* ←	sodium dependent bicarbonate transporter
2,662,727	Δ7 bp	0.000	0.875	0.800	1.000	0.000	0.000	1.000	1.000	coding (360 366/924 nt)	*Synpcc7942_2577* ←	permease of the drug/metabolite transporter

Position	Mutation	SynLE_19	SynLE_20	SynLE_21	SynLE_22	SynLE_23	SynLE_24	Annotation	Gene	Description
25,874	C→A	0.000	0.060	0.000	0.000	0.000	0.000	P153P (CCC→CCA)	*Synpcc7942_0025* →	conserved hypothetical protein
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,060	C→A	0.281	0.165	0.226	0.219	0.110	0.152	G94C (GGC→TGC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	0.837	0.736	0.780	0.735	0.795	0.793	V18F (GTC→TTC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	conserved hypothetical protein
572,999	A→G	0.000	0.000	0.000	0.000	0.000	0.179	noncoding (2376/2878 nt)	*Synpcc7942_R0005* →	23S ribosomal RNA
720,769	G→A	0.056	0.000	0.000	0.000	0.000	0.000	R116H (CGT→CAT)	*Synpcc7942_0727* →	conserved hypothetical protein
802,643	A→G	0.000	0.000	0.000	0.000	0.098	0.000	L113P (CTG→CCG)	*Synpcc7942_0808* ←	HAD superfamily hydrolase subfamily IIB
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	long chain fatty acid CoA ligase
959,499	G→A	0.000	0.000	0.000	0.054	0.000	0.000	A80V (GCG→GTG)	*Synpcc7942_0950* ←	putative multiple sugar transport system substrate binding protein
1,039,086	G→T	0.000	0.000	0.000	0.051	0.000	0.000	Q5K (CAG→AAG)	*Synpcc7942_1024* ←	conserved hypothetical protein
1,044,277	C→A	0.000	0.067	0.000	0.000	0.000	0.000	R112S (CGC→AGC)	*Synpcc7942_1029* →	branched chain amino acid aminotransferase
1,119,786	C→A	0.069	0.000	0.000	0.000	0.000	0.000	Q376K (CAG→AAG)	*Synpcc7942_1101* →	PDZ/DHR/GLGF
1,127,705	G→C	0.069	0.000	0.000	0.000	0.000	0.128	intergenic (+178/+159)	*Synpcc7942_1110* → / ← *Synpcc7942_1111*	response regulator receiver domain protein (CheY like)/serine/threonine protein kinase
1,154,588	T→A	0.000	0.000	0.000	0.056	0.000	0.000	L324F (TTA→TTT)	*Synpcc7942_1133* ←	bacteriocin processing peptidase. Cysteine peptidase. MEROPS family C39
1,238,398	C→T	0.000	0.000	0.000	0.000	0.065	0.000	H285Y (CAT→TAT)	*Synpcc7942_1215* →	acyl CoA dehydrogenase family protein like
1,305,525	G→A	0.056	0.000	0.000	0.000	0.000	0.000	P145S (CCT→TCT)	*Synpcc7942_1283* ←	molybdopterin synthase subunit MoaE
1,422,995	C→A	0.000	0.000	0.000	0.055	0.000	0.000	L39L (CTC→CTA)	*Synpcc7942_1380* →	sulfate permease
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	sodium dependent bicarbonate transporter
1,864,982	G→T	0.000	0.000	0.000	0.052	0.000	0.000	G72V (GGC→GTC)	*Synpcc7942_1794* →	aminotransferase
2,243,434	G→C	0.093	0.000	0.000	0.000	0.000	0.000	W241S (TGG→TCG)	*Synpcc7942_2160* →	alanine glyoxylate aminotransferase
2,314,182	C→A	0.051	0.000	0.000	0.000	0.000	0.000	P625T (CCA→ACA)	*Synpcc7942_2247* →	DNA mismatch repair protein MutS
2,448,715	T→C	0.000	0.053	0.000	0.000	0.000	0.000	L214L (TTG→CTG)	*Synpcc7942_2381* →	3 methyl 2 oxobutanoate hydroxymethyltransferase
2,459,328	G→T	0.000	0.000	0.000	0.000	0.057	0.000	P335Q (CCG→CAG)	*Synpcc7942_2388* ←	Oxalate decarboxylase
2,612,617	C→T	0.000	0.063	0.000	0.000	0.000	0.000	A126V (GCG→GTG)	*Synpcc7942_2531* →	translation elongation factor Ts (EF Ts)
2,655,700	C→G	0.000	0.000	0.000	0.333	0.000	0.000	noncoding (2598/2878 nt)	*Synpcc7942_R0051* ←	23S ribosomal RNA
2,655,707	T→G	0.000	0.000	0.000	0.335	0.000	0.000	noncoding (2591/2878 nt)	*Synpcc7942_R0051* ←	23S ribosomal RNA
2,659,271	T→A	0.000	0.000	0.335	0.000	0.000	0.000	noncoding (1062/1490 nt)	*Synpcc7942_R0052* ←	16S ribosomal RNA
2,662,566	Δ150 bp	0.567	0.467	0.385	0.493	0.530	0.371	coding (378 527/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein
2,662,727	Δ7 bp	1.000	0.942	0.941	1.000	1.000	1.000	coding (360 366/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein

Position	Mutation	SynLL_9	SynLL_10	SynLL_11	SynLL_12	SynLL_13	SynLL_14	SynLL_15	SynLL_16	Annotation	Gene	Description
2,341	A→C	0.000	0.084	0.000	0.000	0.000	0.000	0.000	0.000	N55T (AAC→ACC)	*Synpcc7942_0003* →	two component transcriptional regulator, winged helix family
29,437	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.181	A31T (GCA→ACA)	*Synpcc7942_0028* →	conserved hypothetical protein
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	1.000	1.000	0.797	1.000	1.000	0.941	1.000	1.000	V18F (GTC→TTC)	*Synpcc7942_0095* ←	conserved hypothetical protein
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	conserved hypothetical protein
376,886	T→C	0.000	0.000	0.000	0.000	0.000	0.178	0.000	0.000	S118G (AGC→GGC)	*Synpcc7942_0384* ←	hypothetical protein
388,401	A→C	0.000	0.000	0.000	0.000	0.082	0.000	0.000	0.000	intergenic (181/ 93)	*Synpcc7942_0394* ← / → *Synpcc7942_0395*	phosphatidate cytidylyltransferase/hypothetical protein
477,679	G→T	0.000	0.000	0.000	0.000	0.000	0.171	0.000	0.000	L95F (TTG→TTT)	*Synpcc7942_0490* →	diguanylate cyclase with PAS/PAC sensor
538,847	C→T	0.000	0.000	0.000	0.000	0.097	0.000	0.000	0.000	E125K (GAA→AAA)	*Synpcc7942_0556* ←	two component transcriptional regulator, winged helix family
548,611	T→C	0.076	0.000	0.000	0.000	0.000	0.000	0.000	0.000	D253G (GAC→GGC)	*Synpcc7942_0567* ←	sodium dependent bicarbonate transporter
561,288	G→C	0.000	0.000	0.168	0.000	0.000	0.000	0.000	0.000	A417G (GCG→GGG)	*Synpcc7942_0580* ←	peptidoglycan glycosyltransferase
681,480	A→G	0.000	0.000	0.177	0.000	0.000	0.000	0.000	0.000	E168G (GAG→GGG)	*Synpcc7942_0686* →	FO synthase subunit 2
811,976	T→A	0.000	0.000	0.000	0.000	0.000	0.087	0.000	0.000	Y84F (TAT→TTT)	*Synpcc7942_0816* ←	diguanylate cyclase/phosphodiesterase
851,514	A→G	0.000	0.000	0.119	0.000	0.000	0.000	0.000	0.000	S428G (AGT→GGT)	*Synpcc7942_0854* →	conserved hypothetical protein
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	conserved hypothetical protein
1,110,635	G→C	0.106	0.000	0.000	0.000	0.000	0.000	0.000	0.000	S10C (TCT→TGT)	*Synpcc7942_1092* ←	Glucokinase regulatory like protein
1,189,758	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.180	0.000	D897E (GAC→GAA)	*Synpcc7942_1158* ←	diguanylate cyclase/phosphodiesterase with PAS/PAC and GAF sensor(s)
1,222,004	Δ4 bp	0.102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	coding (392 395/723 nt)	*Synpcc7942_1193* ←	phosphoribosylformylglycinamidine synthase subunit II
1,358,465	G→C	0.000	0.000	0.000	0.116	0.000	0.000	0.000	0.000	D412H (GAC→CAC)	*Synpcc7942_1326* →	transcription repair coupling factor
1,381,026	A→G	0.000	0.000	0.000	0.093	0.000	0.000	0.000	0.000	intergenic (12/+6)	*Synpcc7942_1347* ← / ← *Synpcc7942_1348*	2 hydroxyacid dehydrogenase like/hypothetical protein
1,408,242	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.192	0.000	W376* (TGG→TAG)	*Synpcc7942_1371* ←	magnesium and cobalt transport protein CorA
1,419,895	A→G	0.000	0.000	0.000	0.000	0.000	0.072	0.000	0.000	intergenic (+16/+31)	*Synpcc7942_1377* → / ← *Synpcc7942_1378*	metal dependent phosphohydrolase/conserved hypothetical protein
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	two component transcriptional regulator, winged helix family
1,532,749	T→C	0.000	0.000	0.149	0.000	0.000	0.000	0.000	0.000	S49P (TCC→CCC)	*Synpcc7942_1482* →	conserved hypothetical protein
1,532,792	T→C	0.000	0.000	0.179	0.000	0.000	0.000	0.000	0.000	L63P (CTC→CCC)	*Synpcc7942_1482* →	conserved hypothetical protein
1,628,632	T→C	0.000	0.000	0.000	0.000	0.265	0.000	0.000	0.000	E273G (GAG→GGG)	*Synpcc7942_1570* ←	Heavy metal translocating P type ATPase
1,651,124	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.137	intergenic (138/ 235)	*Synpcc7942_1585* ← / → *Synpcc7942_1586*	N acetylmuramoyl L alanine amidase, family 2/periplasmic sensor signal transduction histidine kinase
1,653,895	T→C	0.000	0.000	0.095	0.000	0.000	0.000	0.000	0.000	E647G (GAG→GGG)	*Synpcc7942_1588* ←	CBS
1,708,946	T→C	0.000	0.000	0.000	0.000	0.000	0.155	0.000	0.000	P94P (CCT→CCC)	*Synpcc7942_1641* →	hypothetical protein
1,903,670	A→G	0.000	0.179	0.000	0.000	0.000	0.000	0.000	0.000	A107A (GCT→GCC)	*Synpcc7942_1836* ←	conserved hypothetical protein
1,964,806	G→T	0.572	0.000	0.000	0.000	0.000	0.000	0.000	0.000	R132L (CGC→CTC)	*Synpcc7942_1891* →	long chain fatty acid CoA ligase
2,091,600	A→T	0.000	0.000	0.000	0.086	0.000	0.000	0.000	0.000	L35* (TTG→TAG)	*Synpcc7942_2022* ←	NusA antitermination factor
2,131,076	A→G	0.000	0.000	0.000	0.149	0.000	0.000	0.000	0.000	D250G (GAT→GGT)	*Synpcc7942_2053* →	probable peptidase
2,168,361	C→T	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (+45/+310)	*Synpcc7942_2088* → / ← *Synpcc7942_2089*	sodium dependent bicarbonate transporter
2,195,560	Δ108 bp	0.000	0.000	0.106	0.000	0.000	0.000	0.000	0.000	coding (734 841/1164 nt)	*Synpcc7942_2114* →	histidine kinase
2,236,807	A→G	0.000	0.000	0.000	0.080	0.000	0.000	0.000	0.000	I279I (ATT→ATC)	*Synpcc7942_2152* ←	conserved hypothetical protein
2,242,759	T→C	0.000	0.000	0.097	0.000	0.000	0.000	0.000	0.000	L16P (CTC→CCC)	*Synpcc7942_2160* →	alanine glyoxylate aminotransferase
2,278,010	G→T	0.000	0.000	0.000	0.104	0.000	0.000	0.000	0.000	S840* (TCG→TAG)	*Synpcc7942_2199* ←	DNA polymerase III, alpha subunit / Intein
2,314,601	C→A	0.000	0.092	0.000	0.000	0.000	0.000	0.000	0.000	R764R (CGC→CGA)	*Synpcc7942_2247* →	conserved hypothetical protein
2,552,403	A→G	0.114	0.000	0.315	0.000	0.000	0.000	0.000	0.000	E236G (GAG→GGG)	*Synpcc7942_2470* →	conserved hypothetical protein/Thioredoxin domain 2
2,639,820	T→C	0.000	0.000	0.000	0.000	0.114	0.000	0.000	0.000	I55V (ATC→GTC)	*Synpcc7942_2561* ←	Delta 9 acyl phospholipid desaturase
2,662,726	Δ1 bp	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.000	coding (367/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein
2,662,727	Δ7 bp	0.909	0.947	1.000	1.000	0.800	0.821	0.933	0.875	coding (360 366/924 nt)	*Synpcc7942_2577* ←	DNA mismatch repair protein MutS
2,692,631	T→C	0.000	0.115	0.000	0.000	0.000	0.000	0.000	0.000	E107G (GAG→GGG)	*Synpcc7942_2610* ←	uroporphyrinogen III C methyltransferase

Position	Mutation	SynLL_1	SynLL_2	SynLL_3	SynLL_4	SynLL_5	SynLL_6	SynLL_8	Annotation	Gene	Description
89,362	G→T	0.000	0.000	0.000	0.000	0.178	0.000	0.000	D210Y (GAT→TAT)	*Synpcc7942_0090* →	transcriptional regulator, GntR family
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	V18F (GTC→TTC)	*Synpcc7942_0095* ←	Rhodanese like
160,741	A→G	0.000	0.000	0.000	0.000	0.106	0.000	0.000	L144P (CTC→CCC)	*Synpcc7942_0158* ←	conserved hypothetical protein
233,422	T→C	0.000	0.000	0.000	0.000	0.116	0.000	0.000	G301G (GGA→GGG)	*Synpcc7942_0238* ←	conserved hypothetical protein
265,601	T→C	0.000	0.000	0.000	0.000	0.204	0.000	0.000	E86G (GAG→GGG)	*Synpcc7942_0269* ←	two component transcriptional regulator, winged helix family
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	putative phage terminase large subunit
518,309	T→C	0.156	0.000	0.000	0.000	0.000	0.000	0.000	R211R (CGA→CGG)	*Synpcc7942_0534* ←	long chain fatty acid CoA ligase
693,111	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.085	I105V (ATC→GTC)	*Synpcc7942_0701* →	conserved hypothetical protein
726,003	A→G	0.000	0.000	0.000	0.000	0.136	0.000	0.000	E177G (GAG→GGG)	*Synpcc7942_0731* →	sulfate permease
823,606	A→T	0.000	0.000	0.097	0.000	0.000	0.000	0.000	Y34F (TAT→TTT)	*Synpcc7942_0829* →	sodium dependent bicarbonate transporter
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	nitrate transport ATP binding subunits C and D
1,047,787	T→C	0.000	0.000	0.000	0.000	0.000	0.078	0.000	C428R (TGC→CGC)	*Synpcc7942_1032* →	RNA methyltransferase TrmH, group 3
1,222,002	Δ1 bp	0.000	0.100	0.000	0.000	0.000	0.000	0.000	coding (397/723 nt)	*Synpcc7942_1193* ←	two component transcriptional regulator, winged helix family
1,222,004	Δ4 bp	0.258	0.100	0.000	0.000	0.308	0.000	0.231	coding (392 395/723 nt)	*Synpcc7942_1193* ←	two component transcriptional regulator, winged helix family
1,222,161	A→G	0.494	0.100	0.000	0.000	0.389	0.000	0.461	S80P (TCC→CCC)	*Synpcc7942_1193* ←	conserved hypothetical protein
1,314,017	A→T	0.000	0.000	0.000	0.000	0.000	0.122	0.000	N669K (AAT→AAA)	*Synpcc7942_1292* ←	DNA topoisomerase I
1,370,873	A→G	0.193	0.100	0.000	0.000	0.000	0.000	0.000	G821G (GGA→GGG)	*Synpcc7942_1337* →	photosystem II D2 protein (photosystem q(a) protein)
1,423,065	A→T	0.000	0.153	0.000	0.000	0.000	0.000	0.000	I63F (ATC→TTC)	*Synpcc7942_1380* →	catalase/peroxidase HPI
1,469,179	G→C	0.000	0.000	0.102	0.000	0.000	0.000	0.000	R279P (CGC→CCC)	*Synpcc7942_1416* →	UDP N acetylmuramate dehydrogenase
1,472,948	G→T	0.106	0.000	0.000	0.000	0.000	0.000	0.000	D122Y (GAT→TAT)	*Synpcc7942_1419* →	conserved hypothetical protein
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	conserved hypothetical protein
1,542,592	A→G	0.000	0.213	0.000	0.000	0.198	0.000	0.000	E592G (GAG→GGG)	*Synpcc7942_1490* →	conserved hypothetical protein
1,726,524	T→C	0.000	0.000	0.103	0.000	0.000	0.000	0.000	S520G (AGC→GGC)	*Synpcc7942_1656* ←	bacterial translation initiation factor 2 (bIF 2)
1,810,793	T→C	0.000	0.000	0.245	0.000	0.000	0.000	0.000	E99G (GAG→GGG)	*Synpcc7942_1740* ←	two component transcriptional regulator, winged helix family
1,825,399	A→G	0.000	0.000	0.000	0.000	0.099	0.000	0.000	L290P (CTC→CCC)	*Synpcc7942_1758* ←	conserved hypothetical protein
1,952,320	C→A	0.000	0.000	0.000	0.000	0.000	0.206	0.000	E90* (GAG→TAG)	*Synpcc7942_1879* ←	LSU ribosomal protein L15P/SSU ribosomal protein S5P
1,964,447	+GATGGTAATAA	0.000	0.000	0.186	0.000	0.000	0.000	0.000	coding (36/561 nt)	*Synpcc7942_1891* →	long chain fatty acid CoA ligase
1,964,485	G→A	0.000	0.000	0.000	0.000	0.000	0.105	0.000	G25D (GGT→GAT)	*Synpcc7942_1891* →	conserved hypothetical protein
1,964,557: 1	+T	0.000	0.000	0.061	0.000	0.000	0.000	0.000	coding (146/561 nt)	*Synpcc7942_1891* →	Glucokinase regulatory like protein
1,964,806	G→T	0.000	0.725	0.446	0.390	0.000	0.631	0.000	R132L (CGC→CTC)	*Synpcc7942_1891* →	sodium dependent bicarbonate transporter
1,964,893	T→G	0.000	0.085	0.143	0.222	0.000	0.253	0.000	L161R (CTG→CGG)	*Synpcc7942_1891* →	conserved hypothetical protein
2,007,653	A→G	0.215	0.000	0.000	0.000	0.000	0.000	0.000	S5G (AGC→GGC)	*Synpcc7942_1934* →	Glucokinase regulatory like protein
2,089,494	A→G	0.000	0.000	0.000	0.000	0.155	0.000	0.000	P148P (CCT→CCC)	*Synpcc7942_2020* ←	two component transcriptional regulator, winged helix family
2,158,496	G→A	0.000	0.000	0.098	0.162	0.000	0.141	0.000	L153L (TTG→TTA)	*Synpcc7942_2080* →	two component transcriptional regulator, winged helix family
2,201,462	G→C	0.000	0.287	0.000	0.000	0.000	0.000	0.000	R65R (CGC→CGG)	*Synpcc7942_2119* ←	long chain fatty acid CoA ligase
2,291,428	G→A	0.000	0.000	0.000	0.000	0.100	0.000	0.000	intergenic (22/+16)	*Synpcc7942_2215* ← / ← *Synpcc7942_2216*	conserved hypothetical protein
2,484,775	A→T	0.000	0.000	0.079	0.000	0.000	0.000	0.000	D159E (GAT→GAA)	*Synpcc7942_2412* ←	DNA primase
2,503,596	T→C	0.000	0.000	0.000	0.000	0.099	0.000	0.000	E787G (GAG→GGG)	*Synpcc7942_2431* ←	sodium dependent bicarbonate transporter
2,507,464	A→G	0.138	0.000	0.000	0.000	0.000	0.000	0.000	S247G (AGC→GGC)	*Synpcc7942_2433* →	MoxR protein like
2,556,812	C→T	0.000	0.000	0.000	0.000	0.095	0.000	0.000	L41L (CTG→TTG)	*Synpcc7942_2475* →	conserved hypothetical protein
2,633,611	T→C	0.140	0.000	0.000	0.000	0.000	0.000	0.000	S13G (AGT→GGT)	*Synpcc7942_2554* ←	conserved hypothetical protein
2,662,727	Δ7 bp	0.879	1.000	0.806	1.000	0.870	0.944	0.927	coding (360 366/924 nt)	*Synpcc7942_2577* ←	conserved hypothetical protein

Position	Mutation	SynLL_17	SynLL_18	SynLL_19	SynLL_20	SynLL_21	SynLL_22	SynLL_23	SynLL_24	Annotation	Gene	Description
30,885	A→G	0.000	0.000	0.137	0.000	0.000	0.000	0.000	0.000	D16G (GAT→GGT)	*Synpcc7942_0030* →	dethiobiotin synthase
51,345	G→T	0.000	0.000	0.000	0.000	0.000	0.134	0.000	0.000	E426* (GAA→TAA)	*Synpcc7942_0050* →	conserved hypothetical protein
52,960	A→G	0.000	0.000	0.000	0.000	0.076	0.000	0.000	0.000	D516D (GAT→GAC)	*Synpcc7942_0051* ←	TPR repeat
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	V18F (GTC→TTC)	*Synpcc7942_0095* ←	conserved hypothetical protein
132,842	T→C	0.000	0.000	0.183	0.000	0.000	0.000	0.000	0.000	D310G (GAC→GGC)	*Synpcc7942_0132* ←	conserved hypothetical protein
199,903	T→C	0.000	0.000	0.000	0.190	0.000	0.000	0.000	0.000	E176G (GAG→GGG)	*Synpcc7942_0198* ←	type 2 NADH dehydrogenase
338,303	A→G	0.000	0.126	0.000	0.000	0.000	0.000	0.000	0.000	L50P (CTC→CCC)	*Synpcc7942_0344* ←	long chain fatty acid CoA ligase
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	sodium dependent bicarbonate transporter
382,629	T→C	0.000	0.000	0.000	0.102	0.000	0.000	0.000	0.000	S276G (AGC→GGC)	*Synpcc7942_0390* ←	Chromate transporter
427,308	G→C	0.000	0.000	0.000	0.061	0.000	0.000	0.000	0.000	intergenic (+158/ 155)	*Synpcc7942_0436* → / → *Synpcc7942_0437*	hypothetical protein/putative glutathione peroxidase
589,398	C→G	0.099	0.000	0.000	0.000	0.000	0.000	0.000	0.000	L221L (CTG→CTC)	*Synpcc7942_0600* ←	conserved hypothetical protein
684,439	T→C	0.000	0.000	0.146	0.000	0.000	0.000	0.000	0.000	E149G (GAG→GGG)	*Synpcc7942_0689* ←	hypothetical protein
738,978	T→C	0.000	0.000	0.000	0.130	0.000	0.000	0.000	0.000	S274P (TCT→CCT)	*Synpcc7942_0744* →	conserved hypothetical protein
803,550	A→T	0.000	0.000	0.000	0.175	0.000	0.000	0.000	0.000	I160F (ATT→TTT)	*Synpcc7942_0809* →	conserved hypothetical protein
826,882	A→G	0.000	0.000	0.000	0.000	0.000	0.107	0.000	0.000	intergenic (877/ 230)	*Synpcc7942_0831* ← / → *Synpcc7942_0833*	conserved hypothetical protein/conserved hypothetical protein
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	conserved hypothetical protein
985,783	C→T	0.000	0.078	0.000	0.000	0.000	0.000	0.000	0.000	A76A (GCG→GCA)	*Synpcc7942_0977* ←	Glucokinase regulatory like protein
1,027,017	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.226	0.000	S467P (TCA→CCA)	*Synpcc7942_1014* ←	CheA signal transduction histidine kinase
1,197,302	G→T	0.000	0.107	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (+810/ 115)	*Synpcc7942_1163* → / → *Synpcc7942_1164*	two component transcriptional regulator, winged helix family
1,214,163	T→C	0.000	0.000	0.137	0.000	0.000	0.000	0.000	0.000	P228P (CCT→CCC)	*Synpcc7942_1183* →	conserved hypothetical protein
1,412,437	C→A	0.000	0.000	0.000	0.086	0.000	0.000	0.000	0.000	Q960K (CAA→AAA)	*Synpcc7942_1372* →	methionine synthase (B12 dependent)
1,443,793	G→A	0.000	0.000	0.000	0.000	0.000	0.185	0.000	0.000	R83H (CGC→CAC)	*Synpcc7942_1394* →	PDZ/DHR/GLGF
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	two component transcriptional regulator, winged helix family
1,532,790	T→C	0.000	0.000	0.000	0.170	0.000	0.000	0.000	0.000	T62T (ACT→ACC)	*Synpcc7942_1482* →	conserved hypothetical protein
1,727,635	T→C	0.000	0.000	0.000	0.000	0.101	0.000	0.000	0.000	A149A (GCA→GCG)	*Synpcc7942_1656* ←	catalase/peroxidase HPI
1,867,792	G→T	0.000	0.000	0.079	0.000	0.000	0.000	0.000	0.000	intergenic (+416/ 72)	*Synpcc7942_1797* → / → *Synpcc7942_1798*	conserved hypothetical protein/conserved hypothetical protein
1,964,447	+GATGGTAATAA	0.000	0.000	0.000	0.000	0.000	0.090	0.000	0.000	coding (36/561 nt)	*Synpcc7942_1891* →	conserved hypothetical protein
1,964,485	G→A	0.090	0.000	0.000	0.000	0.000	0.000	0.000	0.000	G25D (GGT→GAT)	*Synpcc7942_1891* →	conserved hypothetical protein
1,964,557: 1	+T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	coding (146/561 nt)	*Synpcc7942_1891* →	conserved hypothetical protein
1,964,744: 1	+T	0.000	0.000	0.000	0.082	0.000	0.000	0.000	0.000	coding (333/561 nt)	*Synpcc7942_1891* →	
1,964,806	G→T	0.616	0.545	0.443	0.479	0.587	0.376	0.674	0.690	R132L (CGC→CTC)	*Synpcc7942_1891* →	long chain fatty acid CoA ligase
1,964,893	T→G	0.333	0.459	0.277	0.385	0.229	0.306	0.000	0.173	L161R (CTG→CGG)	*Synpcc7942_1891* →	conserved hypothetical protein/conserved hypothetical protein
2,158,496	G→A	0.235	0.421	0.393	0.320	0.343	0.380	0.325	0.140	L153L (TTG→TTA)	*Synpcc7942_2080* →	conserved hypothetical protein
2,179,976	T→A	0.000	0.000	0.000	0.000	0.000	0.000	0.124	0.000	E139V (GAG→GTG)	*Synpcc7942_2097* ←	conserved hypothetical protein
2,208,804	A→T	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	D80V (GAT→GTT)	*Synpcc7942_2130* →	Fructose 6 phosphate phosphoketolase
2,370,611	C→T	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	P24S (CCA→TCA)	*Synpcc7942_2301* →	hypothetical protein
2,392,649	A→G	0.000	0.000	0.000	0.000	0.000	0.144	0.000	0.000	L149S (TTG→TCG)	*Synpcc7942_2325* ←	PBS lyase HEAT like repeat
2,662,727	Δ7 bp	0.903	0.894	0.788	0.900	0.889	0.857	1.000	0.936	coding (360 366/924 nt)	*Synpcc7942_2577* ←	conserved hypothetical protein
2,670,870	G→T	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	S175* (TCA→TAA)	*Synpcc7942_2587* ←	conserved hypothetical protein

Note. Yellow indicates presumed ancestral sweeps. Green indicates those with a probability value above 0.5 but less than 1.0. Dark blue indicates probability values between 0.25 and 0.5. Light blue indicates probability values below 0.2 but above 0.0. These numbers reflect frequency of occurrence of the mutation within each sample. Where 1= 100%.

The qrtPCR data as obtained is shown below.

Table 4. Cycle Threshold (Ct) Values for rtPCR.

w1p1	**w1p2**	**w1p3**	**w1p4**	**w1p5**	**w1p6**	**w1p7**	**w1p8**	**w1p9**	**w1p10**	**w1p11**	**w1p12**
19.75	37.1	18.24	20.01	28.15	17.71	19.97	21.29	18.23	16.41	17.64	20.16
18.89	16.32	18.29	20.83	19.21	17.77	17.63	21.01	19.33	15.22	16.86	18.78
34.12	24.72	16.85	17.99	20.31	19.32	17.81	U	18.84	16.31	18.4	18.59
g1p1	**g1p2**	**g1p3**	**g1p4**	**g1p5**	**g1p6**	**g1p7**	**g1p8**	**g1p9**	**g1p10**	**g1p11**	**g1p12**
19.54	16.64	20.27	19.38	18.04	15.55	U	18.52	18.83	18.7	19.72	18.95
U	16.66	17.97	19.92	18.23	15.78	16.93	18.72	18.57	18.94	19.73	19.81
19.44	16.44	17.9	19.55	19	15.61	16.46	20.26	19.2	18.62	18.95	18.82
o1p1	**o1p2**	**o1p3**	**o1p4**	**o1p5**	**o1p6**	**o1p7**	**o1p8**	**o1p9**	**o1p10**	**o1p11**	**o1p12**
16.44	13.58	28.96	16.5	18.75	15.6	16.78	18.24	18.21	18.15	18.69	18.43
16.54	13.72	15.3	17.8	18.17	15.59	36.95	19.69	18.67	18.74	18.62	18.42
16.96	13.86	15.94	16.6	19.95	16.09	18.16	U	18.21	U	18.84	18.57
no rna p1	**no rna p2**	**no rna p3**	**no rna p4**								
36.94	U	U	U								
U	U	U	36.55								
34.44	30.45	U	35.93								

Note. W, G, and O correspond to white, green, and orange light treatment with sample 1, 2, and 3. P corresponds to primer number 1-4. P1, P5, and P9 are replicates targeting beta carotene. P2, P6, and P10 are replicates targeting Chlorophyll-a. P3, P7, and P11 are replicates targeting phycocyanin. P4, P8, and P12 are replicates targeting the housekeeping gene. Lower Ct values correspond to increased expression, U= undetermined (no readable signal after all cycles).

Figure 31. Raw Data for qrtPCR.

The raw data for the UPLC portion of the study is shown in appendix H.

4.4 Results

Phase 1

We found no hard selective sweeps, where the advantageous mutation has reached fixation, i.e., the probability value equals 1.0, in the genomic data indicating an evolutionary response to the light treatment conditions. We did find five partial soft selective sweeps, where

the advantageous mutation has not reached fixation. However, the sweep mutations that we found occurred mainly in the control group and the orange group. We found, in the control group, soft sweeps for a Δ4 bp and an A→G mutation of Synpcc7942_1193 and a G→T and a T→G mutation of Synpcc7942_1891. We found, in the orange treatment, soft sweeps for a G→T and a T→G mutation of Synpcc7942_1891 and a G→T mutation of Synpcc7942_2080. Therefore, we cannot attribute any of these mutations directly to the light treatments.

The qrtPCR results are summarized in the graphs below.

Figure 32. Ct Values for crtB (beta carotene).

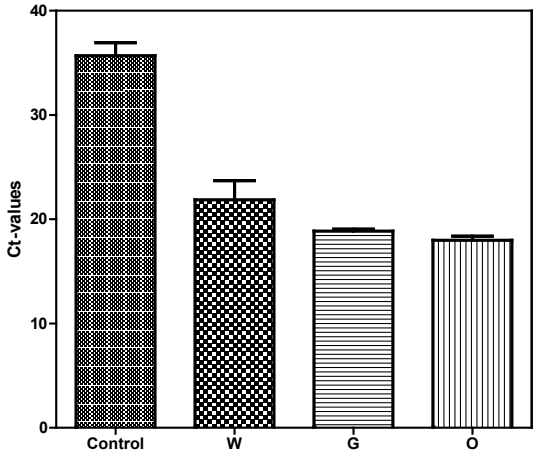


Figure 33. Ct Values for chlG (chlorophyll-a).

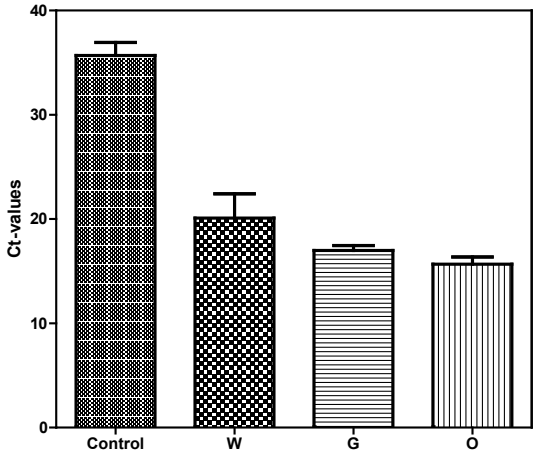


Figure 34. Ct values for cpcB (phycocyanin).

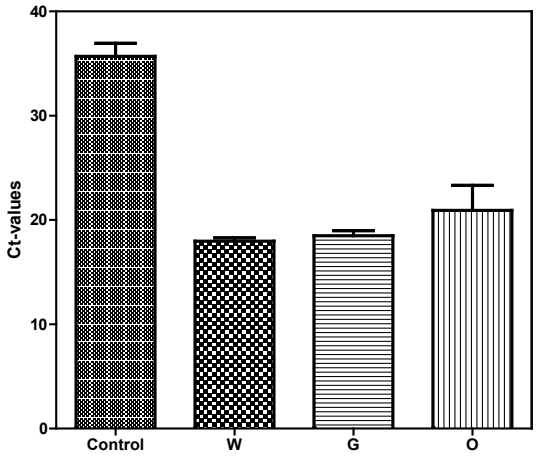
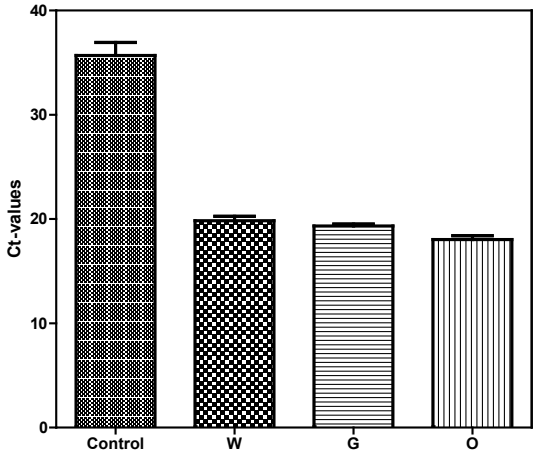


Figure 35. Ct values for ppC (housekeeping gene).



Note. Control (no RNA), white light, green light, and orange light selected samples.

Table 5. Ct Values for Various Pigments and Treatments.

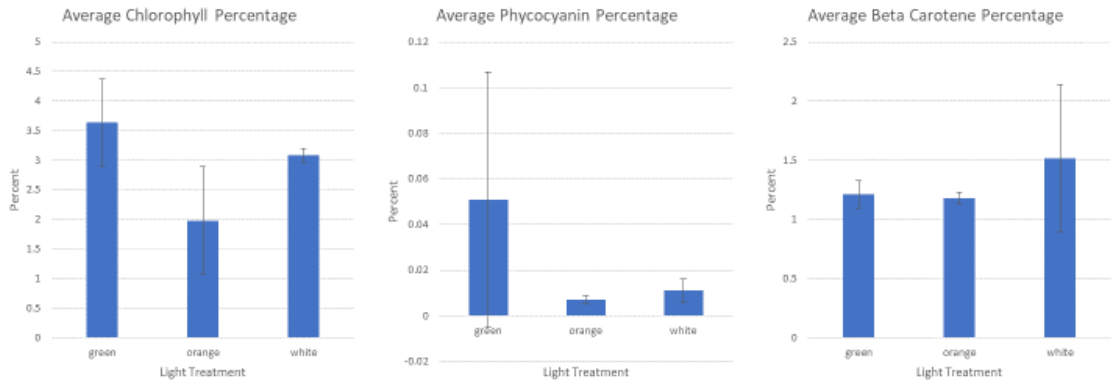
N=9	Beta-carotene	Chlorophyll-a	phycocyanin	HKGene
White	**21.87 ± 10.36**	**20.10 ± 13.13**	**17.97 ± 1.78**	**19.83 ± 2.31**
Green	**18.86 ± 1.02**	**16.99 ± 2.61**	**18.49 ± 2.61**	**19.33 ± 1.14**
Orange	**17.99 ± 2.17**	**15.67 ± 3.69**	**20.92 ± 13.62**	**18.03 ± 1.98**
Control	**35.69 ± 1.25**	**30.45 ± U**	**U**	**36.24 ± 0.31**

In this section, we targeted various genes associated with the production of photosynthetic pigments. We analyzed the expression of these genes through quantitative real time polymerase chain reaction (qrtPCR). Real-time PCR, or qPCR provides a simple and efficient method for determining the amount of a target sequence or gene that is present in a sample (85). The qrtPCR reactions generate a Ct (threshold-cycle) value which is defined as the number of cycles required for the fluorescent signal to cross the threshold, i.e., exceeds

background level. The threshold cycle is inversely proportional to the original relative expression level of the gene of interest. As shown in the above graphs, the orange and green treatments showed a lower Ct value for phytoene synthase than the white light control which indicates an increase in the expression of the gene. They also show a lower Ct value for chlorophyll synthase than the white light control. Interestingly, they show a higher Ct value for phycocyanin subunit beta which indicates less expression of that gene. However, since the Ct value for all treatments was within error for the phycocyanin subunit beta we cannot definitively conclude that there was any effect on its expression due to the narrow band light environments. The Ct values for the housekeeping gene shows relatively similar expression as expected.

The graph below summarizes the concentrations of the various pigments as obtained through UPLC.

Figure 36. Average Percentage of Each Sample Made up of Various Pigments.



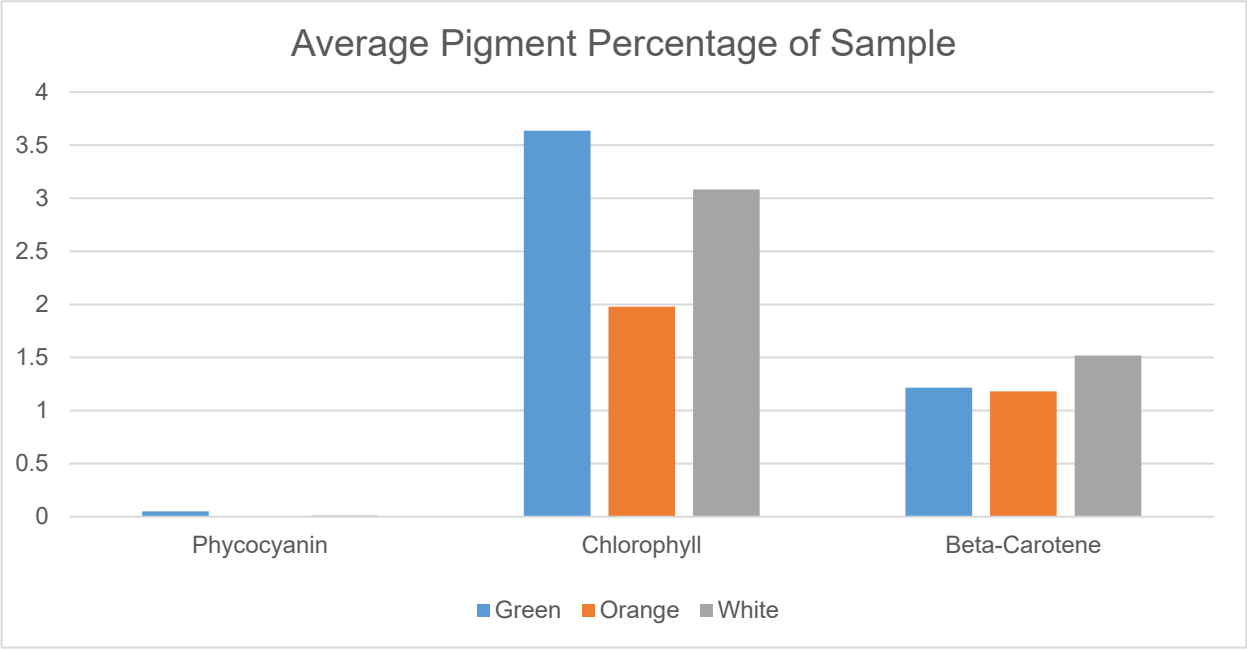


Table 6. Average Percentage of Various Pigments in Each Treatment.

N=3	Beta-carotene	Chlorophyll-a	phycocyanin
White	**1.518 ± 1.242**	**3.083 ± 0.240**	**0.011 ± 0.010**
Green	**1.215 ± 0.232**	**3.637 ± 1.476**	**0.051 ± 0.112**
Orange	**1.181 ± 0.100**	**1.979 ± 1.820**	**0.007 ± 0.004**

While there appears to be changes in the concentrations of chlorophyll-a, phycocyanin, and beta carotene due to the various light treatments, none of them are statistically significant as they fall within the standard deviation error of each other. Chlorophyll-a and phycocyanin show a slightly elevated concentration in green light samples and a slightly decreased concentration in the orange light samples. For beta-carotene, both green and orange light samples show a slight decrease in concentration.

4.5 Discussion

As shown in section 4.4, the genomic results that we acquired showed little support of our initial hypothesis that growing *S. elongatus* in narrow band light environments would lead to mutations in the genome. There were no hard-selective sweeps of any of the mutations that we observed. While many mutations occurred, most were random. The soft selective sweeps that we found in the orange light treatment we also found in the white light control.

Further, utilizing qrtPCR showed us that the expression of our targeted genes in *S. elongatus* was affected by growth in narrow band light environments, as hypothesized. Growth in narrow band light environments, i.e., orange and green, did lead to an increase in the

expression of the targeted genes for both beta carotene and chlorophyll-a with no conclusion able to be drawn about phycocyanin.

Finally, the UPLC results did not definitively support or refute our hypothesis that the concentrations of certain pigments would change based on the light environment in which the samples grew. While we did find that the concentration of the pigments varied between treatment groups, none of the changes were statistically significant. With increased concentration of Chlorophyll-a and phycocyanin in green light and decreased concentration of beta-carotene in green light as well as all pigments in orange light, our results are inconclusive

Table 7. Summary of Predictions and Results for Experiment 3.

Prediction	Result	
narrow band causes genomic changes	No genomic changes	Doesn't support prediction
green will increase beta carotene	increase in b-carotene and chl-a	Supports prediction
orange will increase phycocyanin	no increase in p-cyanin but increase chl-a	Doesn't support prediction
green will increase expression of crtB	no significant change in crtB expression	Doesn't support prediction
orange will increase expression of cpcB	no significant change in cpcB expression	Doesn't support prediction

CHAPTER V: CONCLUSION

5.1 Summary

The research undertaken here explored the effects of fluorescent sensitizers on both a textile plant, *Linum usitatissimum* (Golden Flax), as well as a cyanobacteria, *S. elongatus*. Further, this research explored the effect of narrow band light environments on the photosynthetic cyanobacteria *S. elongatus*. This work elucidated the role of light frequency on photosynthetically driven biomass generation. We hypothesized that the introduction of fluorescent sensitizers, which convert unused portions of the visible spectrum to photosynthetically useful light, would enhance the organism's growth rates and biomass. We further hypothesized that the cyanobacteria grown in narrow band light environments would undergo a genomic response and induce a genetic response that stimulated an increase in various accessory pigments more suitable to absorbing the supplied wavelengths.

The first set of studies involved adding a trans-stilbene dye, D282, as a fluorescent sensitizer that absorbs UV light and emits blue, to *Linum usitatissimum* (Golden Flax). We hypothesized that the experimental group would grow faster and generate more biomass due to additional photosynthetically useful light being available. Specifically, we predicted that the addition of D282 to hydroponically grown flax plants would lead to 1) an increase in the growth rate of the plant crop portion, 2) an increase in the growth rate of the plant root portion, 3) an increase in the leaf surface area and 4) an overall increase in biomass. Additionally, we hypothesized that D282 would not have a negative macroscopic effect on the supplied microbiome due to its reported low toxicity among other organisms. The predictions and results are summarized in table 8 below.

Table 8. Summary of Prediction and Results from Experiment 1.

Prediction	Result	
increase in plant crop growth rate	increased in length between 39%-78%	Supports prediction
increase in plant root growth rate	decreased in length by 63%	Doesn't support prediction
increase in plant leaf growth rate	showed no difference in leaf area	Doesn't support prediction
increase in overall biomass	showed no difference in overall biomass	Doesn't support prediction
no inhibition of microbiome growth rate	showed no difference in growth rate	Supports prediction

The results of this experiment showed a mixed level of support for our predictions. While the increase in crop height supports the hypothesis, the lack of change in leaf surface are and the decrease in root length counteracted that increase and led to a statistically identical overall biomass. This effect on the root growth could be due to competition between the plant and the fluorophore for phosphorous, which is necessary for root formation. Further, the fact that we saw no change in the macroscopic growth rate of the microbiome supports our prediction since the treatment and control groups grew identically.

The second set of experiments we conducted involved the addition of D282 to the growth media of the cyanobacteria *Synechococcus elongatus*. We hypothesized that the experimental group would grow faster and generate more biomass due to additional photosynthetically useful light being available. Further, we hypothesized that the D282 would have an inhibitory or toxic effect at high enough concentrations as is common with any substance. Finally, we hypothesized that at experimental concentrations, D282 would not cause a genomic change to the *S. elongatus* due to its low toxicity among other organisms. The predictions and results are summarized in table 9 below.

Table 9. Summary of Prediction and Results from Experiment 2.

Prediction	Result	
increase in cyanobacteria growth rate	Test grew faster than control	Supports prediction
inhibitory effect on growth rate at high concentration	MIC found at high concentrations	Supports prediction
no mutagenic effect at experimental concentration	no significant mutations	Supports prediction

The results of this experiment tended to support our predictions. The treatment group shows that the additional photosynthetic energy supplied by the fluorophore led to an increase in growth rate for this organism. Further, we found that at a high enough concentration there is an inhibitory effect on the growth of the organism as can be seen, nearly universally, with any additive to a biological system. Finally, our genomic results support our prediction that there would be np mutagenic effect on the organism due to our additive. This comports with its reported low toxicity to other organisms.

The final set of experiments involved the growth of *S. elongatus* in narrow band light environments. We hypothesized that the cyanobacteria would mutate to accommodate for the light environments and increase or decrease the concentrations of various pigments as necessary to thrive. Also, we hypothesized that since each pigment absorbs slightly different wavelengths of visible light, limiting the light to specific wavelengths will lead to an increase in concentration of pigments that absorb those specific wavelengths. Specifically, 1) growth in green light will lead to an increase in beta-carotene production and 2) growth in orange light will lead to an increase in phycocyanin production since those pigments have absorption maxima at those frequencies respectively. Finally, we hypothesized that the expression of genes in the biosynthetic pathway for the production of targeted pigments would be altered. In particular, 1) growth in green light will lead to increased expression of crtB and 2) growth in orange light will lead to an increase in cpcB. The predictions and results are summarized in table 10 below.

Table 10. Summary of Prediction and Results from Experiment 3.

Prediction	Result	
narrow band causes genomic changes	No genomic changes	Doesn't support prediction
green will increase beta carotene	increase in b-carotene and chl-a	Supports prediction
orange will increase phycocyanin	no increase in p-cyanin but increase chl-a	Doesn't support prediction
green will increase expression of crtB	no significant change in crtB expression	Doesn't support prediction
orange will increase expression of cpcB	no significant change in cpcB expression	Doesn't support prediction

The results of this experiment tended to not support our predictions. While we expected a genomic change to account for changes in pigment concentrations due to available light, this is not generally what we found. This implies that there is an internal mechanism to adjust pigments without requiring a genetic change. We found a slight increase in beta carotene in green light but did not see a corresponding increase of phycocyanin in orange light. We did find an increase in chlorophyll-a in both treatments. This may be due to the fact that phycocyanin must be utilized in a phycobiliprotein and it could be more energetically favorable to ramp up chlorophyll production. Finally, the overall expression of the genes responsible for production of our targeted pigments did not change either.

5.2 Future Studies

Some potentially useful future studies could provide further insight into the results seen in these experiments. To begin, for experiment 1. First, introducing the D282 to hydroponically grown flax plants after the roots have firmly established to determine if later application could still provide an increase to the crop portion growth while mitigating the reduction in root growth. Second, growing different crops in the presence of the D282 to determine if the effects seen in Golden Flax are reproduced in other crops. Third, growth of various plants in the presence of other fluorescent sensitizers with properties that could benefit photosynthesis, i.e., absorb portions of the electromagnetic spectrum that are not photosynthetically useful and emit photosynthetically useful light, to determine if they produce similar or better effects than D282.

Fourth, a 16S analysis of the supplied microbiome after exposure to D282 would tell us if the D282 changes the composition of the microbiome. Finally, a full genomic study of the supplied microbiome when grown in the presence of D282 or other optical sensitizers to determine which would be the best at providing positive outcomes to the crop and not negatively impacting the microbiome.

Next, for experiment 2. First, repeating the experiments done here with various photosynthetic bacteria or algae to determine if the same effects can be found universally due to the presence of D282. Second, repeating the experiments done here with various optical sensitizers with properties that could benefit photosynthesis, i.e., absorb portions of the electromagnetic spectrum that are not photosynthetically useful and emit photosynthetically useful light, to determine if they produce similar or better improvements to growth rates than D282 when applied to *S. elongatus* or other photosynthetic bacteria and algae. Third, a directed mutagenesis experiment, carried out for longer than the original experiment, may show where the adaptations seen in the data are affected by D282. Finally, a study of the gene expression profiles of the bacteria in the presence of D282 would elucidate the response caused by the additive.

Finally, for experiment 3. First, repeating the experiments done here with various photosynthetic bacteria or algae to determine if the effects seen are universal across species. Second, repeating the experiments done here but targeting other pigments and genes within *S. elongatus* to see if we can determine if a greater effect can be found in other accessory pigments. Third, a whole genome RNAseq for expression of genes may tell us more about the up or down regulation of pigments. Finally, repeating the experiments done here in various other frequencies

of light to determine if an increased effect on the pigment concentration can be induced by utilizing other narrow band light environments.

This work represents an intriguing start to elucidating the mimicry of accessory pigments to increase the overall growth rate of photoautotrophs as well as their pigment production response to growth in narrow band light. Future studies can help to further increase our depth of knowledge in this area which could potentially lead to methods for increasing yield in agricultural crops, textile crops, and cyanobacteria ponds for conversion to biofuels.

WORKS CITED

1. Buick R (Aug 2008). "When did oxygenic photosynthesis evolve?". Philosophical Transactions of the Royal Society of London, Series B. 363 (1504): 2731–43. doi:10.1098/rstb.2008.0041. PMC 2606769. PMID 18468984

2. Reece J, Urry L, Cain M, Wasserman S, Minorsky P, Jackson R. Biology (International ed.). Upper Saddle River, NJ: Pearson Education. pp. 235, 244. ISBN 0-321-73975-2. “This initial incorporation of carbon into organic compounds is known as carbon fixation.”

3. Moore, R., Clark, W. D., Kingsley, R. S., and Vodopich, D., Botany, Wm. C. Brown, 1995.

4. Armstrong GA, Hearst JE (1996). "Carotenoids 2: Genetics and molecular biology of carotenoid pigment biosynthesis". FASEB J. 10 (2): 228–37. PMID 8641556.

5. Bui, Matthew. "The Arrhenius Law: Activation Energies". Chemistry LibreTexts. UC Davis. Retrieved February 17, 2017.

6. Tipler, Paul (2004). Physics for Scientists and Engineers: Electricity, Magnetism, Light, and Elementary Modern Physics (5th ed.). W. H. Freeman. ISBN 0-7167-0810-8.

7. http://dev.physicslab.org/Document.aspx?doctype=3&filename=AtomicNuclear_Excitation.xml

8. Holler, F. James; Skoog, Douglas A. and Crouch, Stanley R. (2006) Principles Of Instrumental Analysis. Cengage Learning. ISBN 0495012017

9. Cheng, Ping-Chin (2006). "The Contrast Formation in Optical Microscopy". In Pawley, James B. Handbook Of Biological Confocal Microscopy (3rd ed.). New York, NY: Springer. pp. 162–206. doi:10.1007/978-0-387-45524-2_8. ISBN 978-0-387-25921-5.

10. Harris, Daniel C. (2010). "Applications of Spectrophotometry". Quantitative Chemical Analysis (8th ed.). New York: W. H. Freeman and Co. pp. 419–44. ISBN 978-1-4292-1815-3.

11. Helms, Volkhard (2008). "Fluorescence Resonance Energy Transfer". Principles of Computational Cell Biology. Weinheim: Wiley-VCH. p. 202. ISBN 978-3-527-31555-0.

12. Duysens, L. N. M. 1952. Transfer of Excitation Energy in Photosynthesis. Thesis, Univ. of Utrecht.

13. Kevin Padian, Darwin's enduring legacy, Nature 451, 632-634. 7 February, 2008.

14. "Experimental Evolution". Nature.

15. Buckling A, Craig Maclean R, Brockhurst MA, Colegrave N (February 2009). "The Beagle in a bottle". Nature. 457 (7231): 824–9. doi:10.1038/nature07892. PMID 19212400.

16. Elena SF, Lenski RE (June 2003). "Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation". Nat. Rev. Genet. 4 (6): 457–69. doi:10.1038/nrg1088. PMID 12776215

17. Barrick, Jeffrey E.; Lenski, Richard E. "Genome dynamics during experimental evolution". Nature Reviews Genetics. 14 (12): 827–839. doi:10.1038/nrg3564. PMC 4239992 . PMID 24166031.

18. Jha AR, Miles CM, Lippert NR, Brown CD, White KP, Kreitman M (June 2015). "Whole-Genome Resequencing of Experimental Populations Reveals Polygenic Basis of Egg-

Size Variation in Drosophila melanogaster". Mol. Biol. Evol. 32: 2616–32. doi:10.1093/molbev/msv136. PMC 4576704 . PMID 26044351.

19. Turner TL, Stewart AD, et al. (March 2011). "Population-Based Resequencing of Experimentally Evolved Populations Reveals the Genetic Basis of Body Size Variation in Drosophila melanogaster". PLoS Genet. 7: e1001336. doi:10.1371/journal.pgen.1001336. PMC 3060078 . PMID 21437274

20. Ratcliff, WC; Denison, RF; Borrello, M; Travisano, M (31 January 2012). "Experimental evolution of multicellularity.". Proceedings of the National Academy of Sciences of the United States of America. 109 (5): 1595–600. doi:10.1073/pnas.1115323109. PMC 3277146 . PMID 22307617.

21. Barrick, JE; Yu, DS; Yoon, SH; Jeong, H; Oh, TK; Schneider, D; Lenski, RE; Kim, JF (29 October 2009). "Genome evolution and adaptation in a long-term experiment with Escherichia coli.". Nature. 461 (7268): 1243–7. doi:10.1038/nature08480. PMID 19838166.

22. Heineman, RH; Molineux, IJ; Bull, JJ (August 2005). "Evolutionary robustness of an optimal phenotype: re-evolution of lysis in a bacteriophage deleted for its lysin gene.". Journal of Molecular Evolution. 61 (2): 181–91. doi:10.1007/s00239-004-0304-4. PMID 16096681

23. 2010 American Society of Plant Biologists. Early Evolution of Photosynthesis. Robert E. Blankenship*

24. Blankenship RE, Hartman H. (1998) The origin and evolution of oxygenic photosynthesis. Trends Biochem Sci 23: 94–97

25. Des Marais DJ. (2000) Evolution: When did photosynthesis emerge on Earth? Science 289: 1703–1705

26. Buick R. (2008) When did oxygenic photosynthesis evolve? Philos Trans R Soc Lond B Biol Sci 363: 2731–2743

27. Falkowski P, Knoll AN, Blankenship RE, Sadekar S, Raymond J. (2007) The evolutionary transition from anoxygenic to oxygenic photosynthesis. In Falkowski P, Knoll AN, eds, Evolution of Aquatic Photoautotrophs. Academic Press, New York, pp 21–35

28. Raymond J. (2008) Coloring in the tree of life. Trends Microbiol 16: 41–43

29. Margulis L. (1992) Symbiosis in Cell Evolution. WH Freeman, San Francisco

30. Raymond J, Blankenship RE. (2004) Biosynthetic pathways, gene replacement and the antiquity of life. Geobiology 2: 199–203

31. New collaboration aims to address the growing demand for food and fuel by improving the process of photosynthesis. University of Cambridge. 2011.

32. News - Can Scientists Improve Photosynthesis? Science and Plants for Schools. 2017.

33. CAPP: Combining Algal and Plant Photosynthesis. University of Cambridge. 2014

34. Improving Photosynthetic Efficiency for Greater Yield. Annual Review of Plant Biology. Vol. 61:235-261. June 2010.

35. Bernacchi CJ, Portis AR, Nakano H, von Caemmerer S, Long SP. 2002. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. PlantPhysiol. 130:1992–98

36. Dermody O, Long SP, McConnaughay K, DeLucia EH. 2008. How do elevated CO2 and O3 affect the interception and utilization of radiation by a soybean canopy? Glob. Change Biol. 14:556–64

37. Ehleringer J, Pearcy RW. 1983. Variation in quantum yield for CO2 uptake among C3 and C4 plants. PlantPhysiol. 73:555–59

38. Photosynthesis and plant growth at elevated levels of CO2. 1999. Makino, A, Mae, T.

39. Does long-term elevation of CO2 concentration increase photosynthesis in forest floor vegetation?: Indiana strawberry in a Maryland forest. 1997. Osborne, C.P., Drake, B.G., LaRoche, J., Long, S.P.

40. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO2 and temperature. 1998. Tjoelker, M.G., Oleksyn, J., Reich, P.B.

41. Seasonal Changes of Dry Matter Productivity and Photosynthesis in Gerbera hybrid. 2009. Nam, H.H., Woo, J.H., Lee, H.S., Kim, K.U.

42. Environmental influences on photosynthesis and carbon export in greenhouse roses during development of the flowering shoot. 1998. Jiao, J., Grodzinski, B.

43. Leaf temperature effects on net photosynthesis, dark respiration, and photorespiration of seedlings of black locust families with contrasting growth rates. 1991. Mebrahtu, T., Hanover, J.W., Layne, D.R., Flore, J.A.

44. Temperature dependence of growth, development, and photosynthesis in maize under elevated CO_2. 2007., Kim, S.H., Gitz, D.C., Sicher, R.C., Baker, J.T., Timlin, D.J., Reddy, V.R.

45. The effect of growth regulators on photosynthesis and water regime of sugar beet during water stress. 2010. Hejnak, V., Ceska Zemedelska, Katedra Botaniky a Fyziologie Rostlin.

46. Analysis of the effects of diffuse light on photosynthesis and crop production. 2012. Elings, A., Dueck, T., Meinen, E., Kempkes, F.

47. Modelling the effect of diffuse light on canopy photosynthesis in controlled environments. 2002. Cavazzoni, James, Volk, Tyler, Tubiello, Francesco, Monje, Oscar.

48. Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Johannes Kromdijk, Katarzyna Głowacka, Lauriebeth Leonelli, Stéphane T. Gabilly, Masakazu Iwai, Krishna K. Niyogi.

49. "coccus" at Dorland's Medical Dictionary

50. Madigan M; Martinko J, eds. (2005). Brock Biology of Microorganisms (11th ed.). Prentice Hall. ISBN 0-13-144329-1.

51. Salton MRJ, Kim KS (1996). Baron S, et al., eds. Structure. In: Baron's Medical Microbiology (4th ed.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1. (via NCBI Bookshelf).

52. “coccobacillus" Dorlands Medical Dictionary.

53. "Chapter 4: Functional Anatomy Of Prokaryotic And Eukaryotic Cells".

54. Young, Kevin D. (September 2006). "The Selective Value of Bacterial Shape". Microbiology and Molecular Biology Reviews. 70 (3): 660–703.

55. Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations. Rajesh Babu Sekar, Ammasi Periasamy. March 3, 2003.

56. Biochemical characterization and cellular imaging of a novel, membrane permeable fluorescent cAMP analog. Daniela Moll, Anke Prinz, Cornelia M Brendel, Marco Berrera, Katrin Guske, Manuela Zaccolo, Hans-Gottfried Genieser and Friedrich W Herberg. BMC Biochemistry. BioMed Central Ltd. 2008

57. Optimizing fluorescent protein trios for 3-way FRET imaging of protein interactions in living cells. Brandon L. Scott and Adam D. Hoppe. Scientific Reports 5. 2015.

58. Analysis of huntingtin aggregation by fluorescence and FRET microscopy. A. Holloschi, S. Ritz, I. Schäfer, and P. Kioschis. Microscopy: advances in scientific research and education. 2014.

59. Bimolecular Fluorescence Complementation. Katy A. Wong and John P. O'Bryan. Journal of Visualized Experiments. 2011.

60. Fluorescence resonance energy transfer microscopy as demonstrated by measuring the activation of the serine/threonine kinase Akt. Joshua A Broussard, Benjamin Rappaz, Donna J Webb, Claire M Brown. Nature Protocols, 8, 265–281. 2013.

61. Characterization of two-photon excitation fluorescence lifetime imaging microscopy for protein localization. Ye Chen and Ammasi Periasamy. Microscopy Research and Technique. 2003.

62. Wiens JJ. "What is speciation and how should we study it?". American Naturalist. 163 (6): 914–923. 2004.

63. Otto SP. "The evolutionary enigma of sex". American Naturalist. 174 (s1): S1–S14. 2009.

64. Jesse Love Hendrikse, Trish Elizabeth Parsons, Benedikt Hallgrímsson. 2007. "Evolvability as the proper focus of evolutionary developmental biology". Evolution & Development. 9 (4): 393–401.

65. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L; Mardis E; Rotimi CN; Slatkin M; Valle D; Whittemore AS; Boehnke M; Clark AG,

Eichler EE, Gibson G, Haines JL, Mackay TFC, McCarroll SA, Visscher PM. 200. "Finding the missing heritability of complex diseases". Nature. 461 (7265): 747–753.

66. Provine WB. "Progress in evolution and meaning in life". Evolutionary progress. University of Chicago Press. pp. 49–79. 1988.

67. Lenski, Richard E., Rose, Michael R., Simpson, Suzanne C., Tadler, Scott C. 1991. "Long-Term Experimental Evolution in Escherichia coli. I. Adaptation and Divergence During 2,000 Generations". The American Naturalist. 138 (6): 1315–1341.

68. Fox, Jeremy W., Lenski, Richard E. 2015. "From Here to Eternity—The Theory and Practice of a Really Long Experiment". PLOS Biology. 13 (6): e1002185.

69. Blount, Zachary D., Borland, Christina Z., Lenski, Richard E. 2008. "Historical contingency and the evolution of a key innovation in an experimental population of Escherichia coli". Proceedings of the National Academy of Sciences. 105 (23): 7899–7906.

70. Hyman, Paul. "Bacteriophage as instructional organisms in introductory biology labs". Bacteriophage. 4 (2): e27336. 2014.

71. "A Novel Laboratory Activity for Teaching about the Evolution of Multicellularity". The American Biology Teacher. 76 (2): 81–87. 2014.

72. "Using experimental evolution and next-generation sequencing to teach bench and bioinformatic skills". PeerJ PrePrints (3): e1674. 2015.

73. Rapid evolution of silver nanoparticle resistance in Escherichia coli. Joseph L. Graves, Jr., Mehrdad Tajkarimi, Quincy Cunningham, Adero Campbell, Herve Nonga, Scott H. Harrison, and Jeffrey E. Barrick. 2015.

74. Rai M. K., Deshmukh S. D., Ingle A. P., Gade A. K. Silver nanoparticles: the powerful nanoweapon against multi-drug resistant bacteria. J. Appl. Microbiol. 112, 841–852. 2012.

75. Mechanobiology of Antimicrobial Resistant Escherichia coli and Listeria innocua. Mehrdad Tajkarimi, Scott H. Harrison, Albert M. Hung, Joseph L. Graves Jr. 2016.

76. Inventor: Herr, Daniel J.C./UNCG Patent #2016/0044,882 2016 HYDROPONIC COMPOSITIONS AND APPLICATIONS THEREOF

77. Inventor: Herr, Daniel J.C./UNCG, Hydroponic Compositions and Applications Thereof, PCT/US14/32810, File Date: April 3, 2014.

78. Web. sbi4u3.weebly.com 2012

79. http://www.chemistryexplained.com/Ny-Pi/Photosynthesis.html

80. Hye-Ji Kim and Xinxin Li. Effects of Phosphorus on Shoot and Root Growth, Partitioning, and Phosphorus Utilization Efficiency in Lantana. HortScience. https://doi.org/10.21273/HORTSCI.51.8.1001. Page: 1001–1009, Volume 51: Issue 8. Online Publication Date: Aug 2016

81. https://hpvchemicals.oecd.org/UI/handler.axd?id=03A2C013-8BEF-4367-B999-68327F3E7942

82. Xu, Y., Ma, P., Shah, P., Rokas, A., Liu, Y., & Johnson, C. H. (2013). Non-optimal codon usage is a mechanism to achieve circadian clock conditionality. Nature, 495(7439), 116-120.

83. Deatherage, D.E., & Barrick, J. E. , Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. Engineering and Analyzing Multicellular Systems: Methods and Protocols, 2014: p. 165-188.

84. Cao, J., et al., EfeUOB (YcdNOB) is a tripartite, acid-induced and CpxAR-regulated, low-pH Fe2+ transporter that is cryptic in Escherichia coli K-12 but functional in E. coli O157:H7. Mol Microbiol, 2007. 65(4): p. 857-75.

85. Yu, Y., Zhang, G., Chen, Y., Bai, Q., Gao, C., Zeng, L., ... & Guo, L. (2019). Selection of Reference Genes for qPCR Analyses of Gene Expression in Ramie Leaves and Roots across Eleven Abiotic/Biotic Treatments. Scientific reports, 9(1), 1-13.

APPENDIX A: FLAX GROWTH DATA

Bent Stem	T-Test: http://web.mst.edu/~psyworld/texample.htm							1/2 UV	1/2 UV
	102914	110314	110614	110914	111814	120114	120814	121614	122214
Growth	PR Measured						DH Measured		
	102914	110314	110614	110914	111814	120114	120814	121614	122214
Test A1	7.00	7.50	8.00	9.50	11.75	14.00	**17.25**	17.75	19.00
Test A2	9.00	10.50	11.25	14.50	17.25	21.00	26.75	30.75	31.50
Test A3	7.25	9.00	10.00	13.50	17.00	21.25	28.00	29.00	29.25
Test A4	7.25	8.75	10.00	12.50	15.75	17.00	24.75	**27.00**	**29.00**
Test A5	7.25	8.25	9.00	11.50	14.50	16.50	22.00	**23.25**	**26.00**
Test A6	7.50	8.25	9.00	11.00	14.00	17.00	21.13	**22.50**	**24.50**
Test B1	7.00	8.00	9.00	11.50	13.50	15.50	20.50	24.25	27.00
Test B2	7.25	8.50	9.50	12.00	14.25	16.00	22.25	26.25	27.25
Test B3	6.00	7.25	8.00	11.00	13.75	15.00	21.00	23.00	23.75
Test B4	6.50	7.50	8.00	11.00	13.00	15.50	25.00	**23.25**	26.00
Test B5	7.00	7.75	9.00	11.00	13.25	14.75	21.00	23.75	26.75
Test B6	8.00	9.50	10.00	13.50	16.50	18.75	27.50	30.25	30.75
Test C1	6.50	7.50	9.00	11.00	13.50	14.00	21.00	23.25	25.00
Test C2	8.00	9.50	10.50	14.25	17.25	21.00	27.50	28.75	30.50
Test C3	7.00	8.00	9.50	12.00	14.50	17.25	24.00	**23.75**	25.00
Test C4	7.25	8.50	9.25	12.50	15.50	17.75	25.00	28.75	29.75
Test C5	6.00	6.50	7.50	9.00	10.75	12.75	18.00	19.75	22.50
Test C6	6.25	7.75	9.00	12.50	14.50	17.00	19.50	23.50	26.25
Test D1	8.25	10.50	8.50	15.00	14.50	16.75	29.25	29.75	31.75
Test D2	7.00	8.00	9.00	12.25	14.25	17.50	22.00	24.50	27.25
Test D3	8.00	9.75	11.00	14.00	17.00	21.00	26.50	28.75	29.75
Test D4	8.25	10.00	11.25	14.50	18.00	21.50	29.50	**32.25**	**34.25**
Test D5	7.00	7.50	8.25	11.00	13.50	15.00	22.25	**24.50**	**27.25**
Test D6	7.00	7.50	8.50	10.50	12.75	14.00	19.50	**22.25**	**25.25**
Test E1	8.00	8.50	9.25	11.25	13.25	14.75	**18.00**	19.25	21.00
Test E2	6.75	7.25	8.00	9.50	11.50	13.00	16.50	17.25	18.25
Test E3	8.50	9.25	10.25	13.00	15.50	18.00	23.50	26.00	28.75
Test E4	8.00	9.00	10.25	12.50	15.25	17.75	22.25	**25.25**	**27.25**
Test E5	7.50	8.00	9.50	10.50	12.75	14.00	19.75	**21.75**	**24.00**
Test E6	8.00	8.75	10.50	13.00	15.75	18.50	24.00	**27.75**	**29.00**
Mean (N=30)	7.34	8.42	9.33	12.03	14.48	16.79	22.84	24.93	26.78
STDEV(N=30)	0.74	1.00	1.01	1.57	1.82	2.54	3.58	3.83	3.66
							22.93	24.82	26.33
							4.03	4.28	4.30
							22.74	**25.05**	**27.23**
							3.20	**3.46**	**2.96**

	102914	110314	110614	110914	111814	120114	120814	121614	122214	
Cont A1	7.75	9.00	10.00	13.00	15.75	17.00	20.25	**22.00**	**23.00**	
Cont A2	8.00	9.50	10.50	13.00	15.25	17.00	19.50	**20.75**	**22.75**	
Cont A3	7.00	8.25	9.50	11.00	13.00	14.75	17.00	**18.75**	**19.00**	
Cont A4	7.00	7.50	8.50	10.00	12.00	14.00	17.00	17.75	18.75	
Cont A5	7.00	8.00	9.50	11.00	14.25	16.50	17.50	19.50	19.75	
Cont A6	7.25	7.75	9.00	11.00	13.00	15.00	15.75	17.75	20.00	
Cont B1	4.00	4.75	6.00	8.00	9.75	11.50	14.50	**16.00**	**17.50**	
Cont B2	8.25	9.50	10.50	13.00	14.50	15.75	20.50	**22.25**	**24.50**	
Cont B3	7.00	8.50	9.50	13.00	14.75	16.00	20.00	**21.75**	**23.25**	
Cont B4	7.00	8.50	9.00	11.00	14.00	16.50	17.00	17.50	19.00	
Cont B5	6.00	6.50	7.00	8.00	11.25	13.00	**15.75**	17.25	18.50	
Cont B6	4.25	5.50	7.00	9.50	10.50	12.00	12.75	12.50	19.00	
Cont C1	5.25	6.00	7.00	9.00	11.50	13.75	**16.00**	**18.00**	**16.50**	
Cont C2	6.25	7.00	8.00	9.00	10.25	12.00	14.00	**15.25**	**16.00**	
Cont C3	7.00	8.00	9.50	12.00	14.50	17.00	20.50	**21.50**	**23.50**	
Cont C4	5.00	5.00	6.00	8.00	9.00	11.00	13.00	14.00	15.00	
Cont C5	7.00	8.25	9.50	11.50	14.25	15.75	20.00	21.00	23.00	
Cont C6	7.00	8.00	9.00	11.00	12.50	13.75	16.50	17.75	12.00	
Cont D1	7.50	8.00	8.50	10.25	13.00	14.75	**19.00**	**20.25**	**21.75**	
Cont D2	7.50	8.00	8.50	9.50	10.50	12.00	15.00	**16.25**	**17.00**	
Cont D3	7.25	8.25	9.50	12.00	14.50	16.00	20.00	**22.00**	**23.75**	
Cont D4	6.00	7.25	8.50	11.00	13.00	14.75	17.13	18.50	20.75	
Cont D5	8.00	10.00	11.25	14.50	18.00	21.00	24.00	**27.00**	23.00	dying
Cont D6	7.00	7.25	8.00	10.00	11.50	13.75	17.00	18.50	19.75	
Cont E1	9.00	9.50	10.50	13.00	15.50	17.00	22.50	**22.50**	**24.50**	
Cont E2	7.00	7.25	9.50	9.00	11.00	13.00	16.50	**17.75**	**19.00**	
Cont E3	7.25	8.25	9.50	11.25	14.00	16.00	18.75	**20.00**	**19.00**	dying
Cont E4	9.00	9.50	10.50	12.00	14.25	15.00	17.00	16.50	18.75	
Cont E5	7.50	8.75	9.50	11.25	13.25	15.25	15.50	17.00	21.00	
Cont E6	7.00	7.25	8.50	11.00	13.00	14.75	16.00	17.50	19.50	
Mean (N=30)	6.93	7.83	8.91	10.89	13.05	14.85	17.53	18.83	19.96	
STDEV(N=30)	1.16	1.31	1.32	1.67	2.03	2.11	2.68	2.94	2.99	
							18.27	**19.67**	**20.73**	
							2.58	**2.49**	**3.11**	
							16.79	18.00	19.18	
							2.65	3.20	2.76	

TA1-CA6	-0.25	-0.25	-1.00	-1.50	-1.25	-1.00	1.50	0.00
TA2-CA5	2.00	2.50	1.75	3.50	3.00	4.50	9.25	11.25
TA3-CA4	0.25	1.50	1.50	3.50	5.00	7.25	11.00	11.25
TA4-CA3	0.25	0.50	0.50	1.50	2.75	2.25	7.75	8.25
TA5-CA2	-0.75	-1.25	-1.50	-1.50	-0.75	-0.50	2.50	2.50
TA6-CA1	-0.25	-0.75	-1.00	-2.00	-1.75	0.00	0.88	0.50
TB1-CB6	2.75	2.50	2.00	2.00	3.00	3.50	7.75	11.75
TB2-CB5	1.25	2.00	2.50	4.00	3.00	3.00	6.50	9.00
TB3-CB4	-1.00	-1.25	-1.00	0.00	-0.25	-1.50	4.00	5.50
TB4-CB3	-0.50	-1.00	-1.50	-2.00	-1.75	-0.50	5.00	1.50
TB5-CB2	-1.25	-1.75	-1.50	-2.00	-1.25	-1.00	0.50	1.50
TB6-CB1	4.00	4.75	4.00	5.50	6.75	7.25	13.00	14.25
TC1-CC6	-0.50	-0.50	0.00	0.00	1.00	0.25	4.50	5.50
TC2-CC5	1.00	1.25	1.00	2.75	3.00	5.25	7.50	7.75
TC3-CC4	2.00	3.00	3.50	4.00	5.50	6.25	11.00	9.75
TC4-CC3	0.25	0.50	-0.25	0.50	1.00	0.75	4.50	7.25
TC5-CC2	-0.25	-0.50	-0.50	0.00	0.50	0.75	4.00	4.50
TC6-CC1	1.00	1.75	2.00	3.50	3.00	3.25	3.50	5.50
TD1-CD6	1.25	3.25	0.50	5.00	3.00	3.00	12.25	11.25
TD2-CD5	-1.00	-2.00	-2.25	-2.25	-3.75	-3.50	-2.00	-2.50
TD3-CD4	2.00	2.50	2.50	3.00	4.00	6.25	9.38	10.25
TD4-CD3	1.00	1.75	1.75	2.50	3.50	5.50	9.50	10.25
TD5-CD2	-0.50	-0.50	-0.25	1.50	3.00	3.00	7.25	8.25
TD6-CD1	-0.50	-0.50	0.00	0.25	-0.25	-0.75	0.50	2.00
TE1-CE6	1.00	1.25	0.75	0.25	0.25	0.00	2.00	1.75
TE2-CE5	-0.75	-1.50	-1.50	-1.75	-1.75	-2.25	1.00	0.25
TE3-CE4	-0.50	-0.25	-0.25	1.00	1.25	3.00	6.50	9.50
TE4-CE3	0.75	0.75	0.75	1.25	1.25	1.75	3.50	5.25
TE5-CE2	0.50	0.75	0.00	1.50	1.75	1.00	3.25	4.00
TE6-CE1	-1.00	-0.75	0.00	0.00	0.25	1.50	1.50	5.25
M(T-C)	0.41	0.59	0.42	1.13	1.43	1.94	5.31	6.10
STDEV(T-C)	1.26	1.69	1.58	2.25	2.45	2.89	3.92	4.30

	Bent Stem					
	Growth	Container, C (g)	C+Leaves +Stem (g)	C+Leaves (g)	Leaves (g)	Stem (g)
	Test A1	12.95	13.97	13.37	0.42	0.60
	Test A2	12.91	15.92	14.33	1.42	1.59
	Test A3	12.75	16.01	14.08	1.33	1.93
	Test A4	12.93	16.26	14.57	1.64	1.69
	Test A5	12.88	15.86	14.26	1.38	1.60
	Test A6	12.88	15.53	14.17	1.29	1.36
	Test B1	12.93	14.73	13.74	0.81	0.99
	Test B2	12.92	15.19	14.05	1.13	1.14
	Test B3	12.95	15.11	13.93	0.98	1.18
	Test B4	12.86	14.90	13.87	1.01	1.03
	Test B5	12.88	14.91	13.88	1.00	1.03
	Test B6	12.92	16.08	14.51	1.59	1.57
	Test C1	12.91	14.68	13.73	0.82	0.95
	Test C2	12.93	16.09	14.38	1.45	1.71
	Test C3	12.92	15.15	13.92	1.00	1.23
	Test C4	12.91	16.32	14.60	1.69	1.72
	Test C5	12.83	14.31	13.53	0.70	0.78
	Test C6	12.99	15.34	14.12	1.13	1.22
	Test D1	12.89	16.31	14.34	1.45	1.97
	Test D2	12.91	15.08	13.98	1.07	1.10
26leaves=0.33g	Test D3	12.94	16.91	14.88	1.94	2.03
26leaves=0.31g	Test D4	12.76	16.96	14.74	1.98	2.22
	Test D5	12.74	15.14	13.86	1.12	1.28
	Test D6	12.95	15.12	14.04	1.09	1.08
	Test E1	12.94	14.25	13.54	0.60	0.71
	Test E2	12.88	13.93	13.34	0.46	0.59
	Test E3	12.96	15.71	14.16	1.20	1.55
	Test E4	12.96	15.87	14.25	1.29	1.62
	Test E5	12.91	15.32	14.08	1.17	1.24
	Test E6	12.98	16.39	14.65	1.67	1.74
	Mean (N=30)	12.90	15.42	14.09	1.12	1.28
	STDEV(N=30)	0.06	0.79	0.39	0.39	0.42

22leaves=0.26g						
	Cont A1	12.76	16.18	14.34	1.58	1.84
	Cont A2	12.86	15.86	14.38	1.52	1.48
	Cont A3	12.84	15.75	14.12	1.28	1.63
	Cont A4	12.74	14.51	13.66	0.92	0.85
	Cont A5	12.84	14.89	13.77	0.93	1.12
	Cont A6	12.85	14.65	13.66	0.81	0.99
	Cont B1	12.85	14.42	13.71	0.86	0.71
	Cont B2	12.80	19.02	15.92	3.12	3.10
	Cont B3	12.92	17.03	15.02	2.10	2.01
	Cont B4	12.81	14.52	13.71	0.90	0.81
	Cont B5	12.94	14.24	13.57	0.63	0.67
	Cont B6	12.84	13.63	13.21	0.37	0.42
	Cont C1	12.95	14.56	13.69	0.74	0.87
	Cont C2	12.86	15.58	14.43	1.57	1.15
	Cont C3	12.82	16.88	14.86	2.04	2.02
	Cont C4	12.93	13.76	13.39	0.46	0.37
	Cont C5	12.89	16.36	14.64	1.75	1.72
	Cont C6	12.78	16.36	13.77	0.99	2.59
	Cont D1	12.91	15.61	14.13	1.22	1.48
	Cont D2	12.96	17.11	14.97	2.01	2.14
	Cont D3	12.97	16.97	14.95	1.98	2.02
	Cont D4	12.95	14.89	13.90	0.95	0.99
	Cont D5	12.83	14.59	13.75	0.92	0.84
	Cont D6	12.96	14.28	13.59	0.63	0.69
	Cont E1	12.89	18.87	15.76	2.87	3.11
	Cont E2	12.88	15.20	14.02	1.14	1.18
	Cont E3	12.94	15.13	13.93	0.99	1.20
	Cont E4	12.96	14.69	13.79	0.83	0.90
	Cont E5	12.86	17.10	14.64	1.78	2.46
	Cont E6	12.86	14.22	13.47	0.61	0.75
	Mean (N=30)	12.87	15.50	14.14	1.13	1.22
	STDEV(N=30)	0.06	1.36	0.67	0.67	0.74

	no dye biomass (g)	dye biomass (g)	no dye root (in)	dye root (in)
	4.1	4.4	10	10
	6.2	6.3	24	5
	4.4	5.1	10	5
	5.6	5.2	24	5
	5.7	4.4	24	5
	4.24	5.6	10	5
	4.25	4.5	15	5
	5.4	4.1	22	5
	5.87	4.9	20	5
	3.95	5.4	10	5
	3.8	4.8	10	5
	4.1	4.8	10	5
	6.1	4.5	20	5
ave	4.900769231	4.923076923	16.07692308	5.384615385
std dev	0.87492823	0.575331137	6.056929134	1.332346775

APPENDIX B: AZO GROWTH DATA

OD azo 1	OD azo 2	OD azo 3	azo avg	azo median	OD dye 1	OD dye 2	OD dye 3	dye avg	dye median	time (minutes)
0.147	0.134	0.157	0.146	0.147	0.128	0.149	0.132	0.13633	0.132	0
0.266	0.246	0.338	0.283333	0.266	0.247	0.254	0.22	0.24033	0.247	32
0.562	0.503	0.536	0.533667	0.536	0.428	0.504	0.42	0.45067	0.428	57
0.869	0.858	0.966	0.897667	0.869	0.778	0.895	0.766	0.813	0.778	80
1.213	1.127	1.259	1.199667	1.213	1.062	1.198	1.112	1.124	1.112	100
1.543	1.449	1.938	1.643333	1.543	1.407	1.6	1.415	1.474	1.415	133
1.878	1.805	1.996	1.893	1.878	1.752	2.018	1.814	1.86133	1.814	163
2.317	2.183	2.334	2.278	2.317	2.125	2.493	2.128	2.24867	2.128	196
2.688	2.671	2.673	2.677333	2.673	2.624	2.898	2.566	2.696	2.624	229
2.681	2.772	2.873	2.775333	2.772	2.777	3.136	2.769	2.894	2.777	257

OD azo 1	OD azo 2	OD azo 3	azo avg	azo median	OD dye 1	OD dye 2	OD dye 3	dye avg	dye median	time (minutes)
0.123	0.126	0.122	0.123667	0.123	0.124	0.118	0.113	0.118333	0.118	0
0.127	0.143	0.126	0.132	0.127	0.119	0.11	0.116	0.115	0.116	30
0.123	0.127	0.123	0.124333	0.123	0.117	0.134	0.129	0.126667	0.129	60
0.128	0.133	0.131	0.130667	0.131	0.127	0.125	0.13	0.127333	0.127	120
0.138	0.145	0.147	0.143333	0.145	0.144	0.132	0.147	0.141	0.144	152
0.154	0.164	0.162	0.16	0.162	0.153	0.145	0.167	0.155	0.153	177
0.186	0.204	0.2	0.196667	0.2	0.207	0.189	0.211	0.202333	0.207	207
0.232	0.318	0.254	0.268	0.254	0.284	0.476	0.312	0.357333	0.312	234
0.336	0.402	0.375	0.371	0.375	0.405	0.401	0.472	0.426	0.405	266
0.473	0.541	0.499	0.504333	0.499	0.546	0.546	0.674	0.588667	0.546	293
0.689	0.791	0.766	0.748667	0.766	0.818	0.802	0.909	0.843	0.818	324
1.013	1.27	1.109	1.130667	1.109	1.226	1.158	1.291	1.225	1.226	364

OD azo 1	OD azo 2	OD azo 3	azo avg	azo median	OD dye 1	OD dye 2	OD dye 3	dye avg	dye median	time (minutes)
0.374	0.417	0.385	0.392	0.385	0.405	0.371	0.382	0.386	0.382	0
0.389	0.39	0.393	0.390667	0.39	0.408	0.386	0.412	0.402	0.408	32
0.412	0.405	0.457	0.424667	0.412	0.416	0.403	0.415	0.411333	0.415	66
0.447	0.446	0.464	0.452333	0.447	0.474	0.777	0.449	0.566667	0.474	100
0.532	0.526	0.587	0.548333	0.532	0.544	0.552	0.533	0.543	0.544	131
0.784	0.79	0.883	0.819	0.79	0.738	0.742	0.734	0.738	0.738	166
1.04	1.014	1.156	1.07	1.04	0.976	1.042	1.013	1.010333	1.013	196
1.392	1.395	1.529	1.438667	1.395	1.381	1.675	1.412	1.489333	1.412	230
1.783	1.776	1.974	1.844333	1.783	1.72	2.161	1.736	1.872333	1.736	260

APPENDIX C: S. ELONGATUS GROWTH RATE

hour	tr 1	tr 2	tr 3	tr medain	con 1	con 2	con 3	con median
0	0.048	0.053	0.052	0.052	0.049	0.051	0.055	0.051
12	0.062	0.065	0.065	0.065	0.058	0.061	0.063	0.061
24	0.102	0.097	0.085	0.097	0.089	0.092	0.093	0.092
36	0.144	0.152	0.156	0.152	0.143	0.14	0.146	0.143
48	0.218	0.226	0.231	0.226	0.181	0.194	0.191	0.191
72	0.31	0.287	0.318	0.31	0.274	0.271	0.282	0.274
96	0.495	0.481	0.472	0.481	0.386	0.381	0.393	0.386
120	0.722	0.751	0.748	0.748	0.493	0.502	0.51	0.502
144	0.895	0.85	0.876	0.876	0.683	0.718	0.704	0.704
168	0.913	0.921	0.906	0.913	0.848	0.861	0.873	0.861
192	0.964	0.944	0.957	0.957	0.911	0.935	0.928	0.928
	tr medain	error			con median	error		
0	0.052	0.002160247	0.002160247	0	0.051	0.002494438	0.002494438	
12	0.065	0.001414214	0.001414214	12	0.061	0.002054805	0.002054805	
24	0.097	0.007133645	0.007133645	24	0.092	0.001699673	0.001699673	
36	0.152	0.004988877	0.004988877	36	0.143	0.00244949	0.00244949	
48	0.226	0.005354126	0.005354126	48	0.191	0.005557777	0.005557777	
72	0.31	0.013140269	0.013140269	72	0.274	0.004642796	0.004642796	
96	0.481	0.00946338	0.00946338	96	0.386	0.004921608	0.004921608	
120	0.748	0.01302135	0.01302135	120	0.502	0.006944222	0.006944222	
144	0.876	0.018445114	0.018445114	144	0.704	0.014383633	0.014383633	
168	0.913	0.006128259	0.006128259	168	0.861	0.010208929	0.010208929	
192	0.957	0.008286535	0.008286535	192	0.928	0.010077478	0.010077478	

APPENDIX D: S ELONGATUS MIC DATA

T=Treatments										
TO=Blank	Difference in OD									
C=Controls		**4H**								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049366667	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
4H	0.0481	0.0489	0.052833	0.0525	0.052767	0.062967	0.0497	0.057467	0.046233	0.049633
	-0.001266667	**0.0003**	**0.004233**	**0.0048**	**0.003267**	**0.006067**	**0.001833**	**0.004**	**0.003133**	**0.005533**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038266667	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
4H	0.039033333	0.037867	0.037567	0.0384	0.037767	0.037567	0.038633	0.0389	0.0421	0.04
	0.000766667	**3.33E-05**	**-0.00017**	**0.0003**	**0.000167**	**0**	**0.0003**	**0.0004**	**0.003933**	**0.002033**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
4H	0.050333333	0.047	0.047367	0.0493	0.046733	0.047733	0.048233	0.049467	0.048533	0.0519
	-0.002066667	**-0.00327**	**-0.0022**	**-0.0027**	**-0.0025**	**-0.0006**	**-0.00047**	**0.005333**	**0.001833**	**0.007067**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433333	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
4H	0.0527	0.053733	0.056433	0.0557	0.0559	0.056167	0.053967	0.0522	0.0516	0.054367
	0.000266667	**0.006533**	**0.006867**	**0.005633**	**0.008433**	**0.008067**	**0.006533**	**0.003667**	**0.004367**	**0.008567**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
4H	0.0603	0.0611	0.076033	0.058133	0.0567	0.056367	0.054167	0.073133	0.050767	0.0539
	0.0058	**0.0083**	**0.013533**	**0.0084**	**0.007267**	**0.0048**	**0.002667**	**0.016533**	**0.0027**	**0.007533**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233333	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
4H	0.0558	0.050033	0.0518	0.051933	0.048967	0.049733	0.072733	0.049467	0.049267	0.050933
	0.003566667	**0.001833**	**0.002833**	**0.003067**	**0.0022**	**0.000833**	**0.004733**	**0.004**	**0.0069**	**0.009033**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
4H	0.046666667	0.0538	0.044833	0.0459	0.047	0.046767	0.057067	0.0545	0.045767	0.047167
	-0.003833333	**0.000667**	**-0.00047**	**-0.00017**	**0.002**	**0.0027**	**0.0087**	**0.0056**	**0.0036**	**0.005667**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044266667	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
4H	0.0438	0.043467	0.044067	0.044967	0.0439	0.043667	0.044433	0.0458	0.045033	0.046067
	-0.000466667	**-0.00143**	**-0.0001**	**-0.0009**	**0.001333**	**0.0016**	**0.003167**	**0.004367**	**0.004467**	**0.0049**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
4H	0.054033333	0.050267	0.048367	0.048333	0.046767	0.046533	0.045267	0.056333	0.045	0.046067
	0.005333333	**0.004233**	**0.002467**	**0.0036**	**0.0022**	**0.003533**	**0.002467**	**0.0087**	**0.002067**	**0.004367**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046366667	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
4H	0.0449	0.044333	0.0433	0.0455	0.051233	0.053333	0.0442	0.063233	0.043967	0.0456
	-0.001466667	**-0.00033**	**-0.00097**	**0.000433**	**0.000633**	**0.003233**	**0.003267**	**0.0097**	**0.003**	**0.002533**

		8H								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049366667	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
8H	0.052833333	0.051833	0.0531	0.052367	0.052833	0.062867	0.048667	0.060667	0.049733	0.05
	0.003466667	**0.003233**	**0.0045**	**0.004667**	**0.003333**	**0.005967**	**0.0008**	**0.0072**	**0.006633**	**0.0059**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038266667	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
8H	0.039166667	0.038167	0.037433	0.038167	0.037667	0.037433	0.038467	0.038667	0.0412	0.0408
	0.0009	**0.000333**	**-0.0003**	**6.67E-05**	**6.67E-05**	**-0.00013**	**0.000133**	**0.000167**	**0.003033**	**0.002833**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
8H	0.058233333	0.0532	0.0544	0.057767	0.052833	0.0525	0.052733	0.053267	0.053667	0.054833
	0.005833333	**0.002933**	**0.004833**	**0.005767**	**0.0036**	**0.004167**	**0.004033**	**0.009133**	**0.006967**	**0.01**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433333	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
8H	0.060133333	0.055233	0.058767	0.056567	0.057267	0.055933	0.053667	0.0532	0.0538	0.056467
	0.0077	**0.008033**	**0.0092**	**0.0065**	**0.0098**	**0.007833**	**0.006233**	**0.004667**	**0.006567**	**0.010667**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
8H	0.074866667	0.069733	0.091833	0.066667	0.062533	0.060167	0.056367	0.0797	0.056833	0.054033
	0.020366667	**0.016933**	**0.029333**	**0.016933**	**0.0131**	**0.0086**	**0.004867**	**0.0231**	**0.008767**	**0.007667**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233333	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
8H	0.061633333	0.0554	0.057667	0.056367	0.052233	0.0547	0.087867	0.052567	0.054433	0.0536
	0.0094	**0.0072**	**0.0087**	**0.0075**	**0.005467**	**0.0058**	**0.019867**	**0.0071**	**0.012067**	**0.0117**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
8H	0.0494	0.059633	0.047567	0.047167	0.048	0.046567	0.057233	0.056733	0.0473	0.047867
	-0.0011	**0.0065**	**0.002267**	**0.0011**	**0.003**	**0.0025**	**0.008867**	**0.007833**	**0.005133**	**0.006367**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044267	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
8H	0.046333	0.046367	0.0481	0.0489	0.048733	0.048	0.048	0.0475	0.047067	0.0478
	0.002067	**0.001467**	**0.003933**	**0.003033**	**0.006167**	**0.005933**	**0.006733**	**0.006067**	**0.0065**	**0.006633**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
8H	0.0502	0.049933	0.0492	0.047767	0.046433	0.0467	0.045433	0.059533	0.047033	0.0468
	0.0015	**0.0039**	**0.0033**	**0.003033**	**0.001867**	**0.0037**	**0.002633**	**0.0119**	**0.0041**	**0.0051**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
8H	0.047433	0.0454	0.045767	0.045833	0.0553	0.056433	0.0449	0.0683	0.045567	0.0473
	0.001067	**0.000733**	**0.0015**	**0.000767**	**0.0047**	**0.006333**	**0.003967**	**0.014767**	**0.0046**	**0.004233**

		12H								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049367	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
8H	0.057167	0.056267	0.058467	0.057767	0.0592	0.071233	0.0521	0.067467	0.052067	0.0528
	0.0078	**0.007667**	**0.009867**	**0.010067**	**0.0097**	**0.014333**	**0.004233**	**0.014**	**0.008967**	**0.0087**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038267	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
12H	0.0392	0.038733	0.037967	0.0384	0.0382	0.0377	0.0401	0.0388	0.0399	0.042433
	0.000933	**0.0009**	**0.000233**	**0.0003**	**0.0006**	**0.000133**	**0.001767**	**0.0003**	**0.001733**	**0.004467**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
12H	0.075667	0.064467	0.063767	0.068267	0.060367	0.059633	0.057533	0.0598	0.059033	0.0629
	0.023267	**0.0142**	**0.0142**	**0.016267**	**0.011133**	**0.0113**	**0.008833**	**0.015667**	**0.012333**	**0.018067**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
12H	0.071133	0.065533	0.070967	0.0672	0.068667	0.064833	0.062667	0.060833	0.0601	0.063367
	0.0187	**0.018333**	**0.0214**	**0.017133**	**0.0212**	**0.016733**	**0.015233**	**0.0123**	**0.012867**	**0.017567**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
12H	0.0902	0.081367	0.104133	0.075533	0.0691	0.0645	0.059233	0.087433	0.0625	0.059267
	0.0357	**0.028567**	**0.041633**	**0.0258**	**0.019667**	**0.012933**	**0.007733**	**0.030833**	**0.014433**	**0.0129**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
12H	0.0706	0.060667	0.0669	0.0656	0.0609	0.063033	0.102433	0.059133	0.058567	0.057733
	0.018367	**0.012467**	**0.017933**	**0.016733**	**0.014133**	**0.014133**	**0.034433**	**0.013667**	**0.0162**	**0.015833**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
12H	0.051633	0.063767	0.049033	0.0493	0.050167	0.048333	0.0623	0.061533	0.048567	0.047533
	0.001133	**0.010633**	**0.003733**	**0.003233**	**0.005167**	**0.004267**	**0.013933**	**0.012633**	**0.0064**	**0.006033**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044267	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
12H	0.0492	0.049533	0.0513	0.052467	0.051267	0.049933	0.0494	0.048267	0.048467	0.0485
	0.004933	**0.004633**	**0.007133**	**0.0066**	**0.0087**	**0.007867**	**0.008133**	**0.006833**	**0.0079**	**0.007333**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
12H	0.0524	0.053167	0.052533	0.051133	0.049067	0.0486	0.0473	0.060467	0.048833	0.048333
	0.0037	**0.007133**	**0.006633**	**0.0064**	**0.0045**	**0.0056**	**0.0045**	**0.012833**	**0.0059**	**0.006633**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
12H	0.0505	0.050933	0.048667	0.0486	0.065133	0.066567	0.047167	0.0762	0.047467	0.049
	0.004133	**0.006267**	**0.0044**	**0.003533**	**0.014533**	**0.016467**	**0.006233**	**0.022667**	**0.0065**	**0.005933**

		24H								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049367	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
24H	0.068533	0.065467	0.067967	0.066967	0.0685	0.0914	0.058567	0.084667	0.0577	0.0577
	0.019167	**0.016867**	**0.019367**	**0.019267**	**0.019**	**0.0345**	**0.0107**	**0.0312**	**0.0146**	**0.0136**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038267	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
24H	0.039433	0.038533	0.037767	0.038667	0.038233	0.037667	0.039067	0.038433	0.0395	0.0422
	0.001167	**0.0007**	**3.33E-05**	**0.000567**	**0.000633**	**0.0001**	**0.000733**	**-6.7E-05**	**0.001333**	**0.004233**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
24H	0.1001	0.0834	0.079967	0.086433	0.074567	0.0728	0.068	0.071167	0.0699	0.074633
	0.0477	**0.033133**	**0.0304**	**0.034433**	**0.025333**	**0.024467**	**0.0193**	**0.027033**	**0.0232**	**0.0298**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
24H	0.0884	0.078967	0.087067	0.0807	0.0829	0.0755	0.0746	0.070467	0.0698	0.076867
	0.035967	**0.031767**	**0.0375**	**0.030633**	**0.035433**	**0.0274**	**0.027167**	**0.021933**	**0.022567**	**0.031067**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
24H	0.115333	0.102667	0.131033	0.094567	0.0867	0.080267	0.071533	0.112067	0.073567	0.070567
	0.060833	**0.049867**	**0.068533**	**0.044833**	**0.037267**	**0.0287**	**0.020033**	**0.055467**	**0.0255**	**0.0242**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
24H	0.084567	0.070067	0.0759	0.072333	0.0677	0.070133	0.119133	0.069	0.067067	0.0641
	0.032333	**0.021867**	**0.026933**	**0.023467**	**0.020933**	**0.021233**	**0.051133**	**0.023533**	**0.0247**	**0.0222**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
24H	0.058433	0.070567	0.0535	0.053433	0.054767	0.052033	0.072767	0.0701	0.051133	0.050533
	0.007933	**0.017433**	**0.0082**	**0.007367**	**0.009767**	**0.007967**	**0.0244**	**0.0212**	**0.008967**	**0.009033**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044267	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
24H	0.059	0.058533	0.060567	0.060333	0.059467	0.054867	0.054467	0.052567	0.054433	0.055367
	0.014733	**0.013633**	**0.0164**	**0.014467**	**0.0169**	**0.0128**	**0.0132**	**0.011133**	**0.013867**	**0.0142**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
24H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
	-0.00233	**-0.00137**	**-0.00163**	**0.000333**	**0.006033**	**0.0071**	**-0.00187**	**0.0059**	**-0.00197**	**0.001367**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
24H	0.0559	0.055967	0.057667	0.0574	0.078067	0.077367	0.0536	0.096467	0.051733	0.053167
	0.009533	**0.0113**	**0.0134**	**0.012333**	**0.027467**	**0.027267**	**0.012667**	**0.042933**	**0.010767**	**0.0101**

		36H								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049367	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
36H	0.122433	0.111233	0.119667	0.116467	0.120533	0.171333	0.096867	0.164433	0.094267	0.084133
	0.073067	**0.062633**	**0.071067**	**0.068767**	**0.071033**	**0.114433**	**0.049**	**0.110967**	**0.051167**	**0.040033**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038267	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
36H	0.039267	0.038467	0.037867	0.0386	0.0382	0.0377	0.040233	0.0383	0.039633	0.042733
	0.001	**0.000633**	**0.000133**	**0.0005**	**0.0006**	**0.000133**	**0.0019**	**-0.0002**	**0.001467**	**0.004767**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
36H	0.1935	0.152667	0.141867	0.151067	0.129067	0.123433	0.1138	0.1153	0.112	0.106567
	0.1411	**0.1024**	**0.0923**	**0.099067**	**0.079833**	**0.0751**	**0.0651**	**0.071167**	**0.0653**	**0.061733**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
36H	0.180767	0.1592	0.1665	0.155233	0.1598	0.143667	0.148033	0.141033	0.137567	0.1426
	0.128333	**0.112**	**0.116933**	**0.105167**	**0.112333**	**0.095567**	**0.1006**	**0.0925**	**0.090333**	**0.0968**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
36H	0.2211	0.1782	0.2325	0.164667	0.1528	0.1346	0.120833	0.2075	0.1205	0.0927
	0.1666	**0.1254**	**0.17**	**0.114933**	**0.103367**	**0.083033**	**0.069333**	**0.1509**	**0.072433**	**0.046333**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
36H	0.152667	0.1236	0.133467	0.1227	0.1169	0.121	0.213133	0.1177	0.106267	0.088167
	0.100433	**0.0754**	**0.0845**	**0.073833**	**0.070133**	**0.0721**	**0.145133**	**0.072233**	**0.0639**	**0.046267**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
36H	0.095333	0.121367	0.087533	0.085367	0.0866	0.0795	0.128533	0.118267	0.0674	0.058133
	0.044833	**0.068233**	**0.042233**	**0.0393**	**0.0416**	**0.035433**	**0.080167**	**0.069367**	**0.025233**	**0.016633**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044267	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
36H	0.113067	0.1064	0.107833	0.106667	0.104	0.097467	0.090033	0.0756	0.0781	0.076333
	0.0688	**0.0615**	**0.063667**	**0.0608**	**0.061433**	**0.0554**	**0.048767**	**0.034167**	**0.037533**	**0.035167**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
36H	0.0887	0.0864	0.083433	0.079467	0.072867	0.071167	0.0693	0.100033	0.064633	0.061433
	0.04	**0.040367**	**0.037533**	**0.034733**	**0.0283**	**0.028167**	**0.0265**	**0.0524**	**0.0217**	**0.019733**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
36H	0.0975	0.0918	0.0917	0.0948	0.1293	0.1287	0.078733	0.169733	0.0732	0.076067
	0.051133	**0.047133**	**0.047433**	**0.049733**	**0.0787**	**0.0786**	**0.0378**	**0.1162**	**0.032233**	**0.033**

		48H								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049367	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
48H	0.1512	0.138733	0.145733	0.1527	0.154567	0.211133	0.1329	0.202633	0.132433	0.1173
	0.101833	**0.090133**	**0.097133**	**0.105**	**0.105067**	**0.154233**	**0.085033**	**0.149167**	**0.089333**	**0.0732**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038267	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
48H	0.0393	0.039833	0.038433	0.038633	0.038067	0.037733	0.043533	0.038667	0.0397	0.0428
	0.001033	**0.002**	**0.0007**	**0.000533**	**0.000467**	**0.000167**	**0.0052**	**0.000167**	**0.001533**	**0.004833**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
48H	0.230667	0.184933	0.1699	0.179633	0.1562	0.147667	0.137667	0.138933	0.1345	0.133633
	0.178267	**0.134667**	**0.120333**	**0.127633**	**0.106967**	**0.099333**	**0.088967**	**0.0948**	**0.0878**	**0.0888**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
48H	0.216433	0.186133	0.1909	0.181067	0.1913	0.177133	0.176033	0.1763	0.1836	0.194067
	0.164	**0.138933**	**0.141333**	**0.131**	**0.143833**	**0.129033**	**0.1286**	**0.127767**	**0.136367**	**0.148267**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
48H	0.260067	0.2099	0.257733	0.193367	0.1896	0.173267	0.154167	0.2446	0.164167	0.1468
	0.205567	**0.1571**	**0.195233**	**0.143633**	**0.140167**	**0.1217**	**0.102667**	**0.188**	**0.1161**	**0.100433**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
48H	0.198433	0.159433	0.1653	0.153733	0.148867	0.153867	0.248533	0.150533	0.142733	0.138533
	0.1462	**0.111233**	**0.116333**	**0.104867**	**0.1021**	**0.104967**	**0.180533**	**0.105067**	**0.100367**	**0.096633**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
48H	0.112333	0.148067	0.111867	0.110033	0.109067	0.101267	0.156933	0.149233	0.085133	0.074333
	0.061833	**0.094933**	**0.066567**	**0.063967**	**0.064067**	**0.0572**	**0.108567**	**0.100333**	**0.042967**	**0.032833**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044267	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
48H	0.138033	0.105533	0.116567	0.130367	0.128533	0.121533	0.113533	0.097167	0.097667	0.104133
	0.093767	**0.060633**	**0.0724**	**0.0845**	**0.085967**	**0.079467**	**0.072267**	**0.055733**	**0.0571**	**0.062967**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
48H	0.1154	0.107667	0.103133	0.0987	0.0884	0.086167	0.083767	0.126233	0.0782	0.0796
	0.0667	**0.061633**	**0.057233**	**0.053967**	**0.043833**	**0.043167**	**0.040967**	**0.0786**	**0.035267**	**0.0379**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
48H	0.1358	0.1204	0.121	0.124333	0.161733	0.162467	0.100367	0.2097	0.094933	0.108267
	0.089433	**0.075733**	**0.076733**	**0.079267**	**0.111133**	**0.112367**	**0.059433**	**0.156167**	**0.053967**	**0.0652**

		60H								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049367	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
60H	0.262	0.219933	0.221367	0.2273	0.236467	0.319467	0.214133	0.318467	0.218533	0.232233
	0.212633	**0.171333**	**0.172767**	**0.1796**	**0.186967**	**0.262567**	**0.166267**	**0.265**	**0.175433**	**0.188133**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038267	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
60H	0.039333	0.038733	0.037967	0.038767	0.038433	0.037733	0.044867	0.039	0.0394	0.042667
	0.001067	**0.0009**	**0.000233**	**0.000667**	**0.000833**	**0.000167**	**0.006533**	**0.0005**	**0.001233**	**0.0047**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
60H	0.3363	0.268333	0.245967	0.260233	0.237867	0.2223	0.209433	0.213567	0.213633	0.225233
	0.2839	**0.218067**	**0.1964**	**0.208233**	**0.188633**	**0.173967**	**0.160733**	**0.169433**	**0.166933**	**0.1804**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
60H	0.312767	0.2653	0.267	0.2554	0.280567	0.2596	0.269867	0.28	0.2962	0.329767
	0.260333	**0.2181**	**0.217433**	**0.205333**	**0.2331**	**0.2115**	**0.222433**	**0.231467**	**0.248967**	**0.283967**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
60H	0.385933	0.298233	0.3648	0.276833	0.288433	0.2642	0.24	0.368067	0.269367	0.281633
	0.331433	**0.245433**	**0.3023**	**0.2271**	**0.239**	**0.212633**	**0.1885**	**0.311467**	**0.2213**	**0.235267**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
60H	0.311333	0.2455	0.252	0.237167	0.237667	0.241933	0.355733	0.247467	0.240533	0.260867
	0.2591	**0.1973**	**0.203033**	**0.1883**	**0.1909**	**0.193033**	**0.287733**	**0.202**	**0.198167**	**0.218967**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
60H	0.190933	0.206967	0.171933	0.169833	0.1692	0.162	0.238967	0.237667	0.139167	0.1545
	0.140433	**0.153833**	**0.126633**	**0.123767**	**0.1242**	**0.117933**	**0.1906**	**0.188767**	**0.097**	**0.113**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044267	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
60H	0.2367	0.168167	0.178533	0.191067	0.191867	0.1865	0.176867	0.158267	0.1626	0.1951
	0.192433	**0.123267**	**0.134367**	**0.1452**	**0.1493**	**0.144433**	**0.1356**	**0.116833**	**0.122033**	**0.153933**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
60H	0.170067	0.155067	0.148167	0.1461	0.132967	0.131967	0.127167	0.197667	0.121567	0.138433
	0.121367	**0.109033**	**0.102267**	**0.101367**	**0.0884**	**0.088967**	**0.084367**	**0.150033**	**0.078633**	**0.096733**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
60H	0.202167	0.1761	0.1762	0.181367	0.2267	0.231733	0.156	0.303167	0.1539	0.196267
	0.1558	**0.131433**	**0.131933**	**0.1363**	**0.1761**	**0.181633**	**0.115067**	**0.249633**	**0.112933**	**0.1532**

		72H								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049367	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
72H	0.2698	0.226867	0.229533	0.232933	0.2473	0.332433	0.233833	0.340967	0.243333	0.268133
	0.220433	**0.178267**	**0.180933**	**0.185233**	**0.1978**	**0.275533**	**0.185967**	**0.2875**	**0.200233**	**0.224033**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038267	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
72H	0.038467	0.0388	0.0391	0.039133	0.0383	0.0379	0.046533	0.0391	0.039633	0.042867
	0.0002	**0.000967**	**0.001367**	**0.001033**	**0.0007**	**0.000333**	**0.0082**	**0.0006**	**0.001467**	**0.0049**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
72H	0.3602	0.279567	0.257267	0.274967	0.2542	0.2412	0.2343	0.2075	0.201867	0.211367
	0.3078	**0.2293**	**0.2077**	**0.222967**	**0.204967**	**0.192867**	**0.1856**	**0.163367**	**0.155167**	**0.166533**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
72H	0.320667	0.2669	0.269133	0.258733	0.2844	0.2691	0.281533	0.2931	0.3007	0.3239
	0.268233	**0.2197**	**0.219567**	**0.208667**	**0.236933**	**0.221**	**0.2341**	**0.244567**	**0.253467**	**0.2781**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
72H	0.3751	0.296467	0.366933	0.281233	0.2959	0.2787	0.2578	0.3791	0.297233	0.332067
	0.3206	**0.243667**	**0.304433**	**0.2315**	**0.246467**	**0.227133**	**0.2063**	**0.3225**	**0.249167**	**0.2857**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
72H	0.320833	0.253867	0.262333	0.249467	0.253933	0.257733	0.362733	0.2709	0.2667	0.280367
	0.2686	**0.205667**	**0.213367**	**0.2006**	**0.207167**	**0.208833**	**0.294733**	**0.225433**	**0.224333**	**0.238467**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
72H	0.207433	0.221367	0.189167	0.1897	0.191033	0.185167	0.259333	0.263833	0.166767	0.196667
	0.156933	**0.168233**	**0.143867**	**0.143633**	**0.146033**	**0.1411**	**0.210967**	**0.214933**	**0.1246**	**0.155167**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044267	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
72H	0.2342	0.185933	0.2	0.205133	0.2082	0.204467	0.198333	0.183567	0.189933	0.229667
	0.189933	**0.141033**	**0.155833**	**0.159267**	**0.165633**	**0.1624**	**0.157067**	**0.142133**	**0.149367**	**0.1885**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
72H	0.192833	0.162067	0.161467	0.1609	0.1508	0.153	0.148833	0.228767	0.151967	0.2041
	0.144133	**0.116033**	**0.115567**	**0.116167**	**0.106233**	**0.11**	**0.106033**	**0.181133**	**0.109033**	**0.1624**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
72H	0.2278	0.164	0.192633	0.183867	0.235167	0.239333	0.182	0.316033	0.184467	0.205167
	0.181433	**0.119333**	**0.148367**	**0.1388**	**0.184567**	**0.189233**	**0.141067**	**0.2625**	**0.1435**	**0.1621**

		84H								
t5	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.049367	0.0486	0.0486	0.0477	0.0495	0.0569	0.047867	0.053467	0.0431	0.0441
84H	0.3585	0.305667	0.3097	0.324633	0.353133	0.459467	0.351867	0.4827	0.374733	0.426
	0.309133	**0.257067**	**0.2611**	**0.276933**	**0.303633**	**0.402567**	**0.304**	**0.429233**	**0.331633**	**0.3819**
t0	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.038267	0.037833	0.037733	0.0381	0.0376	0.037567	0.038333	0.0385	0.038167	0.037967
84H	0.038433	0.0395	0.038933	0.038767	0.0384	0.0384	0.0471	0.039433	0.0394	0.0427
	0.000167	**0.001667**	**0.0012**	**0.000667**	**0.0008**	**0.000833**	**0.008767**	**0.000933**	**0.001233**	**0.004733**
t3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0524	0.050267	0.049567	0.052	0.049233	0.048333	0.0487	0.044133	0.0467	0.044833
84H	0.351233	0.3452	0.3204	0.350467	0.3388	0.331467	0.3296	0.319767	0.331433	0.370333
	0.298833	**0.294933**	**0.270833**	**0.298467**	**0.289567**	**0.283133**	**0.2809**	**0.275633**	**0.284733**	**0.3255**
t4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052433	0.0472	0.049567	0.050067	0.047467	0.0481	0.047433	0.048533	0.047233	0.0458
84H	0.384133	0.3393	0.3476	0.339033	0.3882	0.370233	0.3982	0.4187	0.4343	0.516267
	0.3317	**0.2921**	**0.298033**	**0.288967**	**0.340733**	**0.322133**	**0.350767**	**0.370167**	**0.387067**	**0.470467**
t1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0545	0.0528	0.0625	0.049733	0.049433	0.051567	0.0515	0.0566	0.048067	0.046367
84H	0.464667	0.3692	0.490633	0.3597	0.400133	0.3765	0.321133	0.478333	0.360467	0.172567
	0.410167	**0.3164**	**0.428133**	**0.309967**	**0.3507**	**0.324933**	**0.269633**	**0.421733**	**0.3124**	**0.1262**
t2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.052233	0.0482	0.048967	0.048867	0.046767	0.0489	0.068	0.045467	0.042367	0.0419
84H	0.3932	0.333067	0.359533	0.344967	0.355667	0.361433	0.484133	0.404333	0.397133	0.395867
	0.340967	**0.284867**	**0.310567**	**0.2961**	**0.3089**	**0.312533**	**0.416133**	**0.358867**	**0.354767**	**0.353967**

C3	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0505	0.053133	0.0453	0.046067	0.045	0.044067	0.048367	0.0489	0.042167	0.0415
84H	0.2476	0.261833	0.238267	0.246967	0.2578	0.2603	0.345967	0.360033	0.2521	0.288433
	0.1971	**0.2087**	**0.192967**	**0.2009**	**0.2128**	**0.216233**	**0.2976**	**0.311133**	**0.209933**	**0.246933**
C4	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.044267	0.0449	0.044167	0.045867	0.042567	0.042067	0.041267	0.041433	0.040567	0.041167
84H	0.300333	0.244133	0.254633	0.260033	0.276833	0.283067	0.281233	0.275333	0.2907	0.328067
	0.256067	**0.199233**	**0.210467**	**0.214167**	**0.234267**	**0.241**	**0.239967**	**0.2339**	**0.250133**	**0.2869**
C1	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.0487	0.046033	0.0459	0.044733	0.044567	0.043	0.0428	0.047633	0.042933	0.0417
84H	0.235167	0.2076	0.203767	0.2132	0.209633	0.221267	0.217067	0.330167	0.227233	0.2799
	0.186467	**0.161567**	**0.157867**	**0.168467**	**0.165067**	**0.178267**	**0.174267**	**0.282533**	**0.1843**	**0.2382**
C2	**0mg/l**	**60.25mg/l**	**120.2mg/l**	**250mg/l**	**500mg/l**	**750mg/l**	**1000mg/l**	**1750mg/l**	**2500mg/l**	**5000mg/l**
0H	0.046367	0.044667	0.044267	0.045067	0.0506	0.0501	0.040933	0.053533	0.040967	0.043067
84H	0.282167	0.215733	0.237767	0.2349	0.307667	0.319533	0.259767	0.4184	0.2787	0.337767
	0.2358	**0.171067**	**0.1935**	**0.189833**	**0.257067**	**0.269433**	**0.218833**	**0.364867**	**0.237733**	**0.2947**

APPENDIX E: RAW GENOMIC DATA EXPERIMENT 2

Position	Mutation	SeT1	SeT2	SeT3	SeT4	SeT5	SeT6	SeT7	SeT8	SeC1	SeC2	SeC3	SeC4	Annotation	Gene	Description
Neutral																
32,861	C→T	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	T114T (ACG→ACA)	*Synpcc7942_0032* ←	Conserved hypothetical protein
878,179	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.088	0.000	G68G (GGC→GGA)	*Synpcc7942_0876* →	alanyl tRNA synthetase
889,961	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.193	0.000	0.000	L322L (CTC→CTA)	*Synpcc7942_0884* ←	translation elongation factor 1A (EF 1A/EF Tu)
903,674	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.090	0.000	F158F (TTC→TTT)	*Synpcc7942_0893* →	photosystem q(b) protein
903,720	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	L174L (TTG→CTG)	*Synpcc7942_0893* →	photosystem q(b) protein
1,361,515	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.150	L117L (CTA→TTA)	*Synpcc7942_1328* →	conserved hypothetical protein
1,371,638	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.096	A1076A (GCA→GCG)	*Synpcc7942_1337* →	Integrins alpha chain
1,435,281	G→A	0.000	0.068	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	L114L (CTG→TTG)	*Synpcc7942_1389* ←	photosystem q(b) protein
1,435,366	A→G	0.000	0.062	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	S85S (TCT→TCC)	*Synpcc7942_1389* ←	photosystem q(b) protein
1,906,685	C→A	0.000	0.000	0.000	0.000	0.244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	V50V (GTG→GTT)	*Synpcc7942_1839* ←	conserved hypothetical protein
1,919,783	T→A	0.000	0.000	0.000	0.140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	G140G (GGT→GGA)	*Synpcc7942_1851* →	Ferredoxin nitrite reductase
Intergenic																
93,449	+T	0.000	0.000	0.000	0.000	0.000	0.075	0.040	0.000	0.000	0.000	0.082	0.000	intergenic (-110/+315)	*Synpcc7942_0095* ← / ← *Synpcc7942_0096*	two component transcriptional regulator, winged helix family/conserved hypothetical protein
1,155,652	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.097	0.000	0.000	intergenic (-93/+2)	*Synpcc7942_1133* ← / ← *Synpcc7942_1134*	bacteriocin processing peptidase. Cysteine peptidase. MEROPS family C39/hypothetical protein
1,157,135	G→A	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (-1173/+278)	*Synpcc7942_1134* ← / ← *Synpcc7942_1135*	hypothetical protein/cation transporter
1,254,576	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.079	0.000	0.000	0.000	0.000	0.000	intergenic (-103/-163)	*Synpcc7942_1233* ← / → *Synpcc7942_1234*	conserved hypothetical protein/conserved hypothetical protein
1,644,608	A→G	0.000	0.000	0.000	0.161	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (-172/-534)	*Synpcc7942_1580* ← / → *Synpcc7942_1581*	hypothetical protein/Peptidase M14, carboxypeptidase A
1,672,074	T→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	intergenic (+63/+49)	*Synpcc7942_1606* → / ← *Synpcc7942_1607*	Beta Ig H3/fasciclin/probable porin; major outer membrane protein
1,672,075	T→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	intergenic (+64/+48)	*Synpcc7942_1606* → / ← *Synpcc7942_1607*	Beta Ig H3/fasciclin/probable porin; major outer membrane protein
1,866,445	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000	intergenic (-2/+11)	*Synpcc7942_1795* ← / ← *Synpcc7942_1796*	SsrA binding protein/conserved hypothetical protein
1,880,779	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	intergenic (+69/-202)	*Synpcc7942_1810* → / → *Synpcc7942_1811*	flavoprotein/diguanylate cyclase (GGDEF domain) with PAS/PAC sensor
2,024,339	C→T	0.089	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (+28/-49)	*Synpcc7942_1949* → / → *Synpcc7942_1950*	conserved hypothetical protein/conserved hypothetical protein
2,596,465	T→C	0.082	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (-6/-119)	*Synpcc7942_2515* ← / → *Synpcc7942_R0018*	putative DNA helicase/tRNA Ala
Indel																
2,662,566	Δ150 bp	0.182	0.209	0.255	0.000	0.000	0.182	0.344	0.203	0.560	0.630	0.639	0.544	coding (378 527/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein
2,662,727	Δ7 bp	0.294	0.385	0.267	0.565	0.462	0.407	0.405	0.623	0.610	0.600	0.500	0.400	coding (360 366/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein

Position	Mutation	SeT1	SeT2	SeT3	SeT4	SeT5	SeT6	SeT7	SeT8	SeC1	SeC2	SeC3	SeC4	Annotation	Gene	Description
SNP																
92,861	G→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.000	T160S (ACT→AGT)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	Two-component transcriptional regulator, winged helix family.
93,060	C→A	0.370	0.265	0.195	0.000	0.319	0.417	0.341	0.249	0.690	0.702	0.637	0.592	G94C (GGC→TGC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	0.670	0.595	0.749	1.000	1.000	0.672	0.684	0.731	0.280	0.203	0.354	0.208	V18F (GTC→TTC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
131,264	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.105	S411I (AGT→ATT)	*Synpcc7942_0131* ←	cyclic nucleotide binding domain (cNMP BD) protein
242,153	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.084	P26L (CCG→CTG)	*Synpcc7942_0245* ←	glyceraldehyde 3 phosphate dehydrogenase
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	conserved hypothetical protein
388,631	T→G	0.000	0.000	0.000	0.000	0.000	0.095	0.000	0.000	0.000	0.000	0.000	0.000	S46R (AGT→AGG)	*Synpcc7942_0395* →	hypothetical protein
487,631	G→A	0.000	0.000	0.000	0.000	0.122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	S247F (TCC→TTC)	*Synpcc7942_0499* ←	hydroxyneurosporene O methyltransferase
617,116	T→C	0.076	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	L233P (CTG→CCG)	*Synpcc7942_0625* →	Single stranded nucleic acid binding R3H
756,548	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000	0.000	S5* (TCA→TAA)	*Synpcc7942_0762* ←	conserved hypothetical protein
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	long chain fatty acid CoA ligase
950,387	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.130	G51W (GGG→TGG)	*Synpcc7942_0943* →	acetylornithine aminotransferase
1,078,628	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.000	R47H (CGC→CAC)	*Synpcc7942_1066* →	hypothetical protein
1,116,847	G→C	0.000	0.000	0.000	0.000	0.000	0.000	0.044	0.000	0.000	0.000	0.000	0.000	A39G (GCC→GGC)	*Synpcc7942_1099* ←	demethylmenaquinone methyltransferase
1,246,732	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	R66W (CGG→TGG)	*Synpcc7942_1225* ←	phycocyanobilin:ferredoxin oxidoreductase
1,370,739	A→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	S777C (AGC→TGC)	*Synpcc7942_1337* →	Integrins alpha chain
1,445,877	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.113	P42T (CCC→ACC)	*Synpcc7942_1396* ←	conserved hypothetical protein
1,458,355	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.051	0.000	0.000	0.000	A100V (GCC→GTC)	*Synpcc7942_1407* →	iron(III) ABC transporter permease protein
1,460,026	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044	0.000	0.000	0.000	0.000	K38R (AAG→AGG)	*Synpcc7942_1408* →	membrane associated protein
1,504,376	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	A26D (GCT→GAT)	*Synpcc7942_1451* →	conserved hypothetical protein
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	sodium dependent bicarbonate transporter
1,536,834	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000	0.000	0.000	Y27H (TAT→CAT)	*Synpcc7942_1486* ←	Protein of unknown function DUF37
1,547,306	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.120	0.000	0.000	0.000	I83V (ATT→GTT)	*Synpcc7942_1496* ←	N acetylglutamate kinase
1,572,744	G→T	0.000	0.000	0.000	0.126	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	A87S (GCT→TCT)	*Synpcc7942_1520* →	SSU ribosomal protein S20P
1,579,838	C→G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.201	0.000	0.000	R249G (CGG→GGG)	*Synpcc7942_1524* →	DNA directed RNA polymerase
1,709,196	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.088	0.000	0.000	0.000	0.000	G6V (GGT→GTT)	*Synpcc7942_1642* →	conserved hypothetical protein
1,786,217	T→A	0.116	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	D480V (GAT→GTT)	*Synpcc7942_1716* ←	diguanylate cyclase/phosphodiesterase with PAS/PAC sensor(s)
1,863,838	T→A	0.000	0.000	0.000	0.000	0.000	0.000	0.052	0.000	0.000	0.000	0.000	0.000	D183E (GAT→GAA)	*Synpcc7942_1792* →	porphobilinogen synthase
2,019,254	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.057	0.000	0.000	0.000	0.000	0.000	R289L (CGC→CTC)	*Synpcc7942_1944* →	Pyruvate dehydrogenase (lipoamide)
2,177,510	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000	R15L (CGG→CTG)	*Synpcc7942_2096* →	diguanylate cyclase with GAF sensor
2,270,158	G→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	V289L (GTT→CTT)	*Synpcc7942_2192* →	diguanylate cyclase (GGDEF domain)
2,375,277	T→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000	S185T (TCA→ACA)	*Synpcc7942_2307* →	conserved hypothetical protein
2,462,070	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000	Q213R (CAA→CGA)	*Synpcc7942_2390* →	5 oxoprolinase (ATP hydrolyzing)
2,514,666	A→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000	0.000	0.000	I131F (ATC→TTC)	*Synpcc7942_2439* →	beta carotene hydroxylase
2,561,810	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.113	0.000	L13F (TTG→TTT)	*Synpcc7942_2480* ←	Prolyl 4 hydroxylase, alpha subunit
2,584,733	A→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.085	0.000	0.000	I243N (ATC→AAC)	*Synpcc7942_2503* ←	chlorophyll synthase / NADPH protochlorophyllide oxidoreductase
2,599,579	G→A	0.092	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	A773V (GCT→GTT)	*Synpcc7942_2519* ←	diguanylate cyclase/phosphodiesterase
2,610,799	T→C	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Q80R (CAA→CGA)	*Synpcc7942_2529* ←	hypothetical protein

APPENDIX F: RAW GENOMIC DATA EARLY-STAGE EXPERIMENT 3

Position	Mutation	SynLE1	SynLE2	SynLE3	SynLE4	SynLE5	SynLE6	SynLE7	SynLE8	SynLE9	SynLE10	Annotation	Gene	Description
78,835	C→A	0.000	0.000	0.000	0.000	0.000	0.253	0.000	0.000	0.000	0.000	V13F (GTC→TTC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
92,978	T→C	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,060	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.137	0.150	G94C (GGC→TGC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.821	0.725	V18F (GTC→TTC)	*Synpcc7942_1183* →	conserved hypothetical protein
118,714	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.160	0.000	intergenic (+287/+21)	*Synpcc7942_0118* → / ← *Synpcc7942_0119*	aspartyl/glutamyl tRNA(Asn/Gln) amidotransferase subunit B/hypothetical protein
194,792	C→A	0.000	0.000	0.000	0.000	0.000	0.190	0.000	0.000	0.000	0.000	R242L (CGC→CTC)	*Synpcc7942_2139* →	probable glutathione S transferase
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_2192* →	diguanylate cyclase (GGDEF domain)
463,267	G→T	0.000	0.000	0.000	0.363	0.000	0.000	0.000	0.000	0.000	0.000	S161R (AGC→AGA)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
538,970	C→G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.129	D84H (GAT→CAT)	*Synpcc7942_0556* ←	two component transcriptional regulator, winged helix family
677,413	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.161	A158S (GCA→TCA)	*Synpcc7942_0683* →	potassium channel protein
688,215	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.114	intergenic (159/+119)	*Synpcc7942_0694* ← / ← *Synpcc7942_0695*	SSU ribosomal protein S1P/Protein of unknown function DUF193
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
1,033,221	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	intergenic (+9/ 79)	*Synpcc7942_1018* → / → *Synpcc7942_1019*	conserved hypothetical protein/4 alpha glucanotransferase
1,213,856	T→C	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	L126S (TTG→TCG)	*Synpcc7942_1415* ←	Proton translocating NADH quinone oxidoreductase, chain N
1,341,107	Δ1 bp	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.148	coding (474/1800 nt)	*Synpcc7942_1313* →	aspartyl tRNA synthetase
1,448,009	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133	0.000	L687L (CTC→CTA)	*Synpcc7942_1398* ←	Cellulose synthase (UDP forming)
1,466,670	C→A	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.000	G476C (GGT→TGT)	*Synpcc7942_1475* ←	sodium dependent bicarbonate transporter
1,526,404	C→T	1.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
1,587,695	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.182	0.000	0.000	0.000	E485* (GAA→TAA)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
1,965,769	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.094	G254G (GGA→GGG)	*Synpcc7942_1893* ←	ATPase
1,965,769	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.094	S267S (AGT→AGC)	*Synpcc7942_1892* →	Rhodanese like
2,070,588	G→T	0.000	0.000	0.211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	G193G (GGC→GGA)	*Synpcc7942_0361* →	conserved hypothetical protein
2,079,723	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.176	0.000	0.000	0.000	A140S (GCC→TCC)	*Synpcc7942_0918* →	long chain fatty acid CoA ligase
2,170,353	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133	T171M (ACG→ATG)	*Synpcc7942_2090* →	homoserine dehydrogenase
2,195,277	T→C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.130	F151L (TTC→CTC)	*Synpcc7942_2114* →	histidine kinase
2,223,689	G→A	0.000	0.000	0.000	0.000	0.252	0.000	0.000	0.000	0.000	0.000	V30I (GTT→ATT)	*Synpcc7942_1475* ←	sodium dependent bicarbonate transporter
2,270,482	G→T	0.000	0.000	0.000	0.191	0.000	0.000	0.000	0.000	0.000	0.000	D397Y (GAT→TAT)	*Synpcc7942_1527* →	nitrogen assimilation regulatory protein
2,416,560	G→T	0.000	0.000	0.000	0.000	0.000	0.222	0.000	0.000	0.000	0.000	E58D (GAG→GAT)	*Synpcc7942_2010* ←	cytochrome c550
2,504,162	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	S598R (AGC→AGA)	*Synpcc7942_2431* ←	conserved hypothetical protein
2,596,739	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.128	E74K (GAG→AAG)	*Synpcc7942_2516* ←	hypothetical protein
2,662,727	Δ7 bp	1.000	0.000	1.000	1.000	0.833	1.000	1.000	1.000	0.857	0.714	coding (360 366/924 nt)	*Synpcc7942_0918* →	long chain fatty acid CoA ligase

Position	Mutation	SynLE_11	SynLE_12	SynLE_13	SynLE_14	SynLE_15	SynLE_16	SynLE_17	SynLE_18	Annotation	Gene	Description
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,060	C→A	0.168	0.278	0.000	0.323	0.364	0.000	0.192	0.179	G94C (GGC→TGC)	*Synpcc7942_0095* ←	Glucokinase regulatory like protein
93,288	C→A	0.759	0.742	0.603	0.636	0.552	1.000	0.797	0.760	V18F (GTC→TTC)	*Synpcc7942_0095* ←	conserved hypothetical protein
166,402	G→T	0.000	0.181	0.000	0.000	0.000	0.000	0.000	0.000	R307L (CGC→CTC)	*Synpcc7942_0166* →	diguanylate cyclase/phosphodiesterase with PAS/PAC and GAF sensor(s)/transcriptional regulator, MarR family
269,799	G→T	0.000	0.000	0.000	0.252	0.000	0.000	0.000	0.000	G48V (GGA→GTA)	*Synpcc7942_0275* →	sodium dependent bicarbonate transporter
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	conserved hypothetical protein
444,444	G→T	0.000	0.000	0.000	0.155	0.000	0.000	0.000	0.000	L148L (CTG→CTT)	*Synpcc7942_0455* →	Glucokinase regulatory like protein
447,878	A→G	0.000	0.105	0.000	0.000	0.000	0.000	0.000	0.000	F97L (TTC→CTC)	*Synpcc7942_0459* ←	two component transcriptional regulator, winged helix family
481,560	C→A	0.000	0.000	0.000	0.000	0.000	0.286	0.000	0.000	I198I (ATC→ATA)	*Synpcc7942_0493* →	conserved hypothetical protein
494,266	A→G	0.000	0.000	0.000	0.105	0.000	0.000	0.000	0.000	K177K (AAA→AAG)	*Synpcc7942_0507* →	two component transcriptional regulator, winged helix family
537,283	T→A	0.000	0.000	0.000	0.145	0.000	0.000	0.000	0.000	N230K (AAT→AAA)	*Synpcc7942_0554* →	conserved hypothetical protein
643,728	G→T	0.000	0.000	0.159	0.000	0.000	0.000	0.000	0.000	A80D (GCT→GAT)	*Synpcc7942_0649* ←	queuine tRNA ribosyltransferase
722,372	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.051	0.000	R650R (CGG→CGA)	*Synpcc7942_0727* →	ribosome recycling factor
793,466	G→T	0.000	0.000	0.000	0.000	0.235	0.000	0.000	0.000	R309S (CGT→AGT)	*Synpcc7942_0799* ←	long chain fatty acid CoA ligase
848,508	C→A	0.000	0.000	0.087	0.000	0.000	0.000	0.000	0.000	intergenic (+250/ 362)	*Synpcc7942_0851* → / → *Synpcc7942_0853*	nitrate transport ATP binding subunits C and D
860,868	G→C	0.154	0.000	0.000	0.000	0.000	0.000	0.000	0.000	A251P (GCT→CCT)	*Synpcc7942_0859* →	stage II sporulation protein D like
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	Glucokinase regulatory like protein
1,127,640	C→A	0.285	0.000	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (+113/+224)	*Synpcc7942_1110* → / ← *Synpcc7942_1111*	two component transcriptional regulator, winged helix family
1,192,766	G→T	0.000	0.000	0.122	0.000	0.000	0.000	0.000	0.000	intergenic (318/+26)	*Synpcc7942_1158* ← / ← *Synpcc7942_1159*	two component transcriptional regulator, winged helix family
1,202,173	G→T	0.000	0.191	0.000	0.000	0.000	0.000	0.000	0.000	P565T (CCT→ACT)	*Synpcc7942_1169* ←	Elongator protein 3
1,259,833	G→A	0.000	0.000	0.000	0.166	0.000	0.000	0.000	0.000	L375L (CTC→CTT)	*Synpcc7942_1237* ←	pyridine nucleotide transhydrogenase beta subunit
1,342,945	G→A	0.000	0.000	0.156	0.000	0.000	0.000	0.000	0.000	A487A (GCC→GCT)	*Synpcc7942_1314* ←	long chain fatty acid CoA ligase
1,393,137	C→A	0.160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	D1212Y (GAC→TAC)	*Synpcc7942_1357* ←	Glucokinase regulatory like protein
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	two component transcriptional regulator, winged helix family
1,585,991	G→T	0.000	0.000	0.148	0.000	0.000	0.000	0.000	0.000	E235D (GAG→GAT)	*Synpcc7942_1526* →	conserved hypothetical protein
1,680,129	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.000	V87V (GTG→GTT)	*Synpcc7942_1610* ←	tRNA i(6)A37 thiotransferase enzyme MiaB
1,710,882	Δ3 bp	0.000	0.130	0.000	0.000	0.000	0.000	0.000	0.000	coding (530 532/1521 nt)	*Synpcc7942_1643* →	conserved hypothetical protein
1,882,490	T→C	0.000	0.000	0.126	0.000	0.000	0.000	0.000	0.000	S504P (TCA→CCA)	*Synpcc7942_1811* →	long chain fatty acid CoA ligase
1,992,607	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	W80* (TGG→TAG)	*Synpcc7942_1917* →	sodium dependent bicarbonate transporter
1,998,975	T→A	0.000	0.000	0.134	0.000	0.000	0.000	0.000	0.000	Q61L (CAG→CTG)	*Synpcc7942_1922* ←	two component transcriptional regulator, winged helix family
2,081,946	G→A	0.000	0.000	0.000	0.145	0.000	0.000	0.000	0.000	W147* (TGG→TGA)	*Synpcc7942_2012* →	two component transcriptional regulator, winged helix family
2,164,639	G→T	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	intergenic (+43/+12)	*Synpcc7942_2083* → / ← *Synpcc7942_2084*	two component transcriptional regulator, winged helix family
2,442,754	C→A	0.000	0.000	0.000	0.000	0.000	0.000	0.052	0.000	R436R (CGC→CGA)	*Synpcc7942_2374* →	conserved hypothetical protein
2,648,802	G→T	0.159	0.000	0.000	0.000	0.000	0.000	0.000	0.000	R7S (CGC→AGC)	*Synpcc7942_2569* ←	long chain fatty acid CoA ligase
2,662,566	Δ150 bp	0.000	0.641	0.651	0.638	0.709	0.000	0.416	0.256	coding (378 527/924 nt)	*Synpcc7942_2577* ←	sodium dependent bicarbonate transporter
2,662,727	Δ7 bp	0.000	0.875	0.800	1.000	0.000	0.000	1.000	1.000	coding (360 366/924 nt)	*Synpcc7942_2577* ←	permease of the drug/metabolite transporter

Position	Mutation	SynLE_19	SynLE_20	SynLE_21	SynLE_22	SynLE_23	SynLE_24	Annotation	Gene	Description
25,874	C→A	0.000	0.060	0.000	0.000	0.000	0.000	P153P (CCC→CCA)	*Synpcc7942_0025* →	conserved hypothetical protein
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,060	C→A	0.281	0.165	0.226	0.219	0.110	0.152	G94C (GGC→TGC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	0.837	0.736	0.780	0.735	0.795	0.793	V18F (GTC→TTC)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	conserved hypothetical protein
572,999	A→G	0.000	0.000	0.000	0.000	0.000	0.179	noncoding (2376/2878 nt)	*Synpcc7942_R0005* →	23S ribosomal RNA
720,769	G→A	0.056	0.000	0.000	0.000	0.000	0.000	R116H (CGT→CAT)	*Synpcc7942_0727* →	conserved hypothetical protein
802,643	A→G	0.000	0.000	0.000	0.000	0.098	0.000	L113P (CTG→CCG)	*Synpcc7942_0808* ←	HAD superfamily hydrolase subfamily IIB
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	long chain fatty acid CoA ligase
959,499	G→A	0.000	0.000	0.000	0.054	0.000	0.000	A80V (GCG→GTG)	*Synpcc7942_0950* ←	putative multiple sugar transport system substrate binding protein
1,039,086	G→T	0.000	0.000	0.000	0.051	0.000	0.000	Q5K (CAG→AAG)	*Synpcc7942_1024* ←	conserved hypothetical protein
1,044,277	C→A	0.000	0.067	0.000	0.000	0.000	0.000	R112S (CGC→AGC)	*Synpcc7942_1029* →	branched chain amino acid aminotransferase
1,119,786	C→A	0.069	0.000	0.000	0.000	0.000	0.000	Q376K (CAG→AAG)	*Synpcc7942_1101* →	PDZ/DHR/GLGF
1,127,705	G→C	0.069	0.000	0.000	0.000	0.000	0.128	intergenic (+178/+159)	*Synpcc7942_1110* → / ← *Synpcc7942_1111*	response regulator receiver domain protein (CheY like)/serine/threonine protein kinase
1,154,588	T→A	0.000	0.000	0.000	0.056	0.000	0.000	L324F (TTA→TTT)	*Synpcc7942_1133* ←	bacteriocin processing peptidase. Cysteine peptidase. MEROPS family C39
1,238,398	C→T	0.000	0.000	0.000	0.000	0.065	0.000	H285Y (CAT→TAT)	*Synpcc7942_1215* →	acyl CoA dehydrogenase family protein like
1,305,525	G→A	0.056	0.000	0.000	0.000	0.000	0.000	P145S (CCT→TCT)	*Synpcc7942_1283* ←	molybdopterin synthase subunit MoaE
1,422,995	C→A	0.000	0.000	0.000	0.055	0.000	0.000	L39L (CTC→CTA)	*Synpcc7942_1380* →	sulfate permease
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	sodium dependent bicarbonate transporter
1,864,982	G→T	0.000	0.000	0.000	0.052	0.000	0.000	G72V (GGC→GTC)	*Synpcc7942_1794* →	aminotransferase
2,243,434	G→C	0.093	0.000	0.000	0.000	0.000	0.000	W241S (TGG→TCG)	*Synpcc7942_2160* →	alanine glyoxylate aminotransferase
2,314,182	C→A	0.051	0.000	0.000	0.000	0.000	0.000	P625T (CCA→ACA)	*Synpcc7942_2247* →	DNA mismatch repair protein MutS
2,448,715	T→C	0.000	0.053	0.000	0.000	0.000	0.000	L214L (TTG→CTG)	*Synpcc7942_2381* →	3 methyl 2 oxobutanoate hydroxymethyltransferase
2,459,328	G→T	0.000	0.000	0.000	0.000	0.057	0.000	P335Q (CCG→CAG)	*Synpcc7942_2388* ←	Oxalate decarboxylase
2,612,617	C→T	0.000	0.063	0.000	0.000	0.000	0.000	A126V (GCG→GTG)	*Synpcc7942_2531* →	translation elongation factor Ts (EF Ts)
2,655,700	C→G	0.000	0.000	0.000	0.333	0.000	0.000	noncoding (2598/2878 nt)	*Synpcc7942_R0051* ←	23S ribosomal RNA
2,655,707	T→G	0.000	0.000	0.000	0.335	0.000	0.000	noncoding (2591/2878 nt)	*Synpcc7942_R0051* ←	23S ribosomal RNA
2,659,271	T→A	0.000	0.000	0.335	0.000	0.000	0.000	noncoding (1062/1490 nt)	*Synpcc7942_R0052* ←	16S ribosomal RNA
2,662,566	Δ150 bp	0.567	0.467	0.385	0.493	0.530	0.371	coding (378 527/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein
2,662,727	Δ7 bp	1.000	0.942	0.941	1.000	1.000	1.000	coding (360 366/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein

APPENDIX G: RAW GENOMIC DATA LATE-STAGE EXPERIMENT 3

Position	Mutation	SynLL_1	SynLL_2	SynLL_3	SynLL_4	SynLL_5	SynLL_6	SynLL_8	Annotation	Gene	Description
89,362	G→T	0.000	0.000	0.000	0.000	0.178	0.000	0.000	D210Y (GAT→TAT)	*Synpcc7942_0090* →	transcriptional regulator, GntR family
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	V18F (GTC→TTC)	*Synpcc7942_0095* ←	Rhodanese like
160,741	A→G	0.000	0.000	0.000	0.000	0.106	0.000	0.000	L144P (CTC→CCC)	*Synpcc7942_0158* ←	conserved hypothetical protein
233,422	T→C	0.000	0.000	0.000	0.000	0.116	0.000	0.000	G301G (GGA→GGG)	*Synpcc7942_0238* ←	conserved hypothetical protein
265,601	T→C	0.000	0.000	0.000	0.000	0.204	0.000	0.000	E86G (GAG→GGG)	*Synpcc7942_0269* ←	two component transcriptional regulator, winged helix family
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	putative phage terminase large subunit
518,309	T→C	0.156	0.000	0.000	0.000	0.000	0.000	0.000	R211R (CGA→CGG)	*Synpcc7942_0534* ←	long chain fatty acid CoA ligase
693,111	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.085	I105V (ATC→GTC)	*Synpcc7942_0701* →	conserved hypothetical protein
726,003	A→G	0.000	0.000	0.000	0.000	0.136	0.000	0.000	E177G (GAG→GGG)	*Synpcc7942_0731* →	sulfate permease
823,606	A→T	0.000	0.000	0.097	0.000	0.000	0.000	0.000	Y34F (TAT→TTT)	*Synpcc7942_0829* →	sodium dependent bicarbonate transporter
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	nitrate transport ATP binding subunits C and D
1,047,787	T→C	0.000	0.000	0.000	0.000	0.000	0.078	0.000	C428R (TGC→CGC)	*Synpcc7942_1032* →	RNA methyltransferase TrmH, group 3
1,222,002	Δ1 bp	0.000	0.100	0.000	0.000	0.000	0.000	0.000	coding (397/723 nt)	*Synpcc7942_1193* ←	two component transcriptional regulator, winged helix family
1,222,004	Δ4 bp	0.258	0.100	0.000	0.000	0.308	0.000	0.231	coding (392 395/723 nt)	*Synpcc7942_1193* ←	two component transcriptional regulator, winged helix family
1,222,161	A→G	0.494	0.100	0.000	0.000	0.389	0.000	0.461	S80P (TCC→CCC)	*Synpcc7942_1193* ←	conserved hypothetical protein
1,314,017	A→T	0.000	0.000	0.000	0.000	0.000	0.122	0.000	N669K (AAT→AAA)	*Synpcc7942_1292* ←	DNA topoisomerase I
1,370,873	A→G	0.193	0.100	0.000	0.000	0.000	0.000	0.000	G821G (GGA→GGG)	*Synpcc7942_1337* →	photosystem II D2 protein (photosystem q(a) protein)
1,423,065	A→T	0.000	0.153	0.000	0.000	0.000	0.000	0.000	I63F (ATC→TTC)	*Synpcc7942_1380* →	catalase/peroxidase HPI
1,469,179	G→C	0.000	0.000	0.102	0.000	0.000	0.000	0.000	R279P (CGC→CCC)	*Synpcc7942_1416* →	UDP N acetylmuramate dehydrogenase
1,472,948	G→T	0.106	0.000	0.000	0.000	0.000	0.000	0.000	D122Y (GAT→TAT)	*Synpcc7942_1419* →	conserved hypothetical protein
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	conserved hypothetical protein
1,542,592	A→G	0.000	0.213	0.000	0.000	0.198	0.000	0.000	E592G (GAG→GGG)	*Synpcc7942_1490* →	conserved hypothetical protein
1,726,524	T→C	0.000	0.000	0.103	0.000	0.000	0.000	0.000	S520G (AGC→GGC)	*Synpcc7942_1656* ←	bacterial translation initiation factor 2 (bIF 2)
1,810,793	T→C	0.000	0.000	0.245	0.000	0.000	0.000	0.000	E99G (GAG→GGG)	*Synpcc7942_1740* ←	two component transcriptional regulator, winged helix family
1,825,399	A→G	0.000	0.000	0.000	0.000	0.099	0.000	0.000	L290P (CTC→CCC)	*Synpcc7942_1758* ←	conserved hypothetical protein
1,952,320	C→A	0.000	0.000	0.000	0.000	0.000	0.206	0.000	E90* (GAG→TAG)	*Synpcc7942_1879* ←	LSU ribosomal protein L15P/SSU ribosomal protein S5P
1,964,447	+GATGGTAATAA	0.000	0.000	0.186	0.000	0.000	0.000	0.000	coding (36/561 nt)	*Synpcc7942_1891* →	long chain fatty acid CoA ligase
1,964,485	G→A	0.000	0.000	0.000	0.000	0.000	0.105	0.000	G25D (GGT→GAT)	*Synpcc7942_1891* →	conserved hypothetical protein
1,964,557: 1	+T	0.000	0.000	0.061	0.000	0.000	0.000	0.000	coding (146/561 nt)	*Synpcc7942_1891* →	Glucokinase regulatory like protein
1,964,806	G→T	0.000	0.725	0.446	0.390	0.000	0.631	0.000	R132L (CGC→CTC)	*Synpcc7942_1891* →	sodium dependent bicarbonate transporter
1,964,893	T→G	0.000	0.085	0.143	0.222	0.000	0.253	0.000	L161R (CTG→CGG)	*Synpcc7942_1891* →	conserved hypothetical protein
2,007,653	A→G	0.215	0.000	0.000	0.000	0.000	0.000	0.000	S5G (AGC→GGC)	*Synpcc7942_1934* →	Glucokinase regulatory like protein
2,089,494	A→G	0.000	0.000	0.000	0.000	0.155	0.000	0.000	P148P (CCT→CCC)	*Synpcc7942_2020* ←	two component transcriptional regulator, winged helix family
2,158,496	G→A	0.000	0.000	0.098	0.162	0.000	0.141	0.000	L153L (TTG→TTA)	*Synpcc7942_2080* →	two component transcriptional regulator, winged helix family
2,201,462	G→C	0.000	0.287	0.000	0.000	0.000	0.000	0.000	R65R (CGC→CGG)	*Synpcc7942_2119* ←	long chain fatty acid CoA ligase
2,291,428	G→A	0.000	0.000	0.000	0.000	0.100	0.000	0.000	intergenic (22/+16)	*Synpcc7942_2215* ← / ← *Synpcc7942_2216*	conserved hypothetical protein
2,484,775	A→T	0.000	0.000	0.079	0.000	0.000	0.000	0.000	D159E (GAT→GAA)	*Synpcc7942_2412* ←	DNA primase
2,503,596	T→C	0.000	0.000	0.000	0.000	0.099	0.000	0.000	E787G (GAG→GGG)	*Synpcc7942_2431* ←	sodium dependent bicarbonate transporter
2,507,464	A→G	0.138	0.000	0.000	0.000	0.000	0.000	0.000	S247G (AGC→GGC)	*Synpcc7942_2433* →	MoxR protein like
2,556,812	C→T	0.000	0.000	0.000	0.000	0.095	0.000	0.000	L41L (CTG→TTG)	*Synpcc7942_2475* →	conserved hypothetical protein
2,633,611	T→C	0.140	0.000	0.000	0.000	0.000	0.000	0.000	S13G (AGT→GGT)	*Synpcc7942_2554* ←	conserved hypothetical protein
2,662,727	Δ7 bp	0.879	1.000	0.806	1.000	0.870	0.944	0.927	coding (360 366/924 nt)	*Synpcc7942_2577* ←	conserved hypothetical protein

Position	Mutation	SynLL_9	SynLL_10	SynLL_11	SynLL_12	SynLL_13	SynLL_14	SynLL_15	SynLL_16	Annotation	Gene	Description
2,341	A→C	0.000	0.084	0.000	0.000	0.000	0.000	0.000	0.000	N55T (AAC→ACC)	*Synpcc7942_0003* →	two component transcriptional regulator, winged helix family
29,437	G→A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.181	A31T (GCA→ACA)	*Synpcc7942_0028* →	conserved hypothetical protein
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	1.000	1.000	0.797	1.000	1.000	0.941	1.000	1.000	V18F (GTC→TTC)	*Synpcc7942_0095* ←	conserved hypothetical protein
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	conserved hypothetical protein
376,886	T→C	0.000	0.000	0.000	0.000	0.000	0.178	0.000	0.000	S118G (AGC→GGC)	*Synpcc7942_0384* ←	hypothetical protein
388,401	A→C	0.000	0.000	0.000	0.000	0.082	0.000	0.000	0.000	intergenic (181/ 93)	*Synpcc7942_0394* ← / → *Synpcc7942_0395*	phosphatidate cytidylyltransferase/hypothetical protein
477,679	G→T	0.000	0.000	0.000	0.000	0.000	0.171	0.000	0.000	L95F (TTG→TTT)	*Synpcc7942_0490* →	diguanylate cyclase with PAS/PAC sensor
538,847	C→T	0.000	0.000	0.000	0.000	0.097	0.000	0.000	0.000	E125K (GAA→AAA)	*Synpcc7942_0556* ←	two component transcriptional regulator, winged helix family
548,611	T→C	0.076	0.000	0.000	0.000	0.000	0.000	0.000	0.000	D253G (GAC→GGC)	*Synpcc7942_0567* ←	sodium dependent bicarbonate transporter
561,288	G→C	0.000	0.000	0.168	0.000	0.000	0.000	0.000	0.000	A417G (GCG→GGG)	*Synpcc7942_0580* ←	peptidoglycan glycosyltransferase
681,480	A→G	0.000	0.000	0.177	0.000	0.000	0.000	0.000	0.000	E168G (GAG→GGG)	*Synpcc7942_0686* →	FO synthase subunit 2
811,976	T→A	0.000	0.000	0.000	0.000	0.000	0.087	0.000	0.000	Y84F (TAT→TTT)	*Synpcc7942_0816* ←	diguanylate cyclase/phosphodiesterase
851,514	A→G	0.000	0.000	0.119	0.000	0.000	0.000	0.000	0.000	S428G (AGT→GGT)	*Synpcc7942_0854* →	conserved hypothetical protein
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	conserved hypothetical protein
1,110,635	G→C	0.106	0.000	0.000	0.000	0.000	0.000	0.000	0.000	S10C (TCT→TGT)	*Synpcc7942_1092* ←	Glucokinase regulatory like protein
1,189,758	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.180	0.000	D897E (GAC→GAA)	*Synpcc7942_1158* ←	diguanylate cyclase/phosphodiesterase with PAS/PAC and GAF sensor(s)
1,222,004	Δ4 bp	0.102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	coding (392 395/723 nt)	*Synpcc7942_1193* ←	phosphoribosylformylglycinamidine synthase subunit II
1,358,465	G→C	0.000	0.000	0.000	0.116	0.000	0.000	0.000	0.000	D412H (GAC→CAC)	*Synpcc7942_1326* →	transcription repair coupling factor
1,381,026	A→G	0.000	0.000	0.000	0.093	0.000	0.000	0.000	0.000	intergenic (12/+6)	*Synpcc7942_1347* ← / ← *Synpcc7942_1348*	2 hydroxyacid dehydrogenase like/hypothetical protein
1,408,242	C→T	0.000	0.000	0.000	0.000	0.000	0.000	0.192	0.000	W376* (TGG→TAG)	*Synpcc7942_1371* ←	magnesium and cobalt transport protein CorA
1,419,895	A→G	0.000	0.000	0.000	0.000	0.000	0.072	0.000	0.000	intergenic (+16/+31)	*Synpcc7942_1377* → / ← *Synpcc7942_1378*	metal dependent phosphohydrolase/conserved hypothetical protein
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	two component transcriptional regulator, winged helix family
1,532,749	T→C	0.000	0.000	0.149	0.000	0.000	0.000	0.000	0.000	S49P (TCC→CCC)	*Synpcc7942_1482* →	conserved hypothetical protein
1,532,792	T→C	0.000	0.000	0.179	0.000	0.000	0.000	0.000	0.000	L63P (CTC→CCC)	*Synpcc7942_1482* →	conserved hypothetical protein
1,628,632	T→C	0.000	0.000	0.000	0.000	0.265	0.000	0.000	0.000	E273G (GAG→GGG)	*Synpcc7942_1570* ←	Heavy metal translocating P type ATPase
1,651,124	G→T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.137	intergenic (138/ 235)	*Synpcc7942_1585* ← / → *Synpcc7942_1586*	N acetylmuramoyl L alanine amidase, family 2/periplasmic sensor signal transduction histidine kinase
1,653,895	T→C	0.000	0.000	0.095	0.000	0.000	0.000	0.000	0.000	E647G (GAG→GGG)	*Synpcc7942_1588* ←	CBS
1,708,946	T→C	0.000	0.000	0.000	0.000	0.000	0.155	0.000	0.000	P94P (CCT→CCC)	*Synpcc7942_1641* →	hypothetical protein
1,903,670	A→G	0.000	0.179	0.000	0.000	0.000	0.000	0.000	0.000	A107A (GCT→GCC)	*Synpcc7942_1836* ←	conserved hypothetical protein
1,964,806	G→T	0.572	0.000	0.000	0.000	0.000	0.000	0.000	0.000	R132L (CGC→CTC)	*Synpcc7942_1891* →	long chain fatty acid CoA ligase
2,091,600	A→T	0.000	0.000	0.000	0.086	0.000	0.000	0.000	0.000	L35* (TTG→TAG)	*Synpcc7942_2022* ←	NusA antitermination factor
2,131,076	A→G	0.000	0.000	0.000	0.149	0.000	0.000	0.000	0.000	D250G (GAT→GGT)	*Synpcc7942_2053* →	probable peptidase
2,168,361	C→T	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (+45/+310)	*Synpcc7942_2088* → / ← *Synpcc7942_2089*	sodium dependent bicarbonate transporter
2,195,560	Δ108 bp	0.000	0.000	0.106	0.000	0.000	0.000	0.000	0.000	coding (734 841/1164 nt)	*Synpcc7942_2114* →	histidine kinase
2,236,807	A→G	0.000	0.000	0.000	0.080	0.000	0.000	0.000	0.000	I279I (ATT→ATC)	*Synpcc7942_2152* ←	conserved hypothetical protein
2,242,759	T→C	0.000	0.000	0.097	0.000	0.000	0.000	0.000	0.000	L16P (CTC→CCC)	*Synpcc7942_2160* →	alanine glyoxylate aminotransferase
2,278,010	G→T	0.000	0.000	0.000	0.104	0.000	0.000	0.000	0.000	S840* (TCG→TAG)	*Synpcc7942_2199* ←	DNA polymerase III, alpha subunit / Intein
2,314,601	C→A	0.000	0.092	0.000	0.000	0.000	0.000	0.000	0.000	R764R (CGC→CGA)	*Synpcc7942_2247* →	conserved hypothetical protein
2,552,403	A→G	0.114	0.000	0.315	0.000	0.000	0.000	0.000	0.000	E236G (GAG→GGG)	*Synpcc7942_2470* →	conserved hypothetical protein/Thioredoxin domain 2
2,639,820	T→C	0.000	0.000	0.000	0.000	0.114	0.000	0.000	0.000	I55V (ATC→GTC)	*Synpcc7942_2561* ←	Delta 9 acyl phospholipid desaturase
2,662,726	Δ1 bp	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.000	coding (367/924 nt)	*Synpcc7942_2577* ←	Glucokinase regulatory like protein
2,662,727	Δ7 bp	0.909	0.947	1.000	1.000	0.800	0.821	0.933	0.875	coding (360 366/924 nt)	*Synpcc7942_2577* ←	DNA mismatch repair protein MutS
2,692,631	T→C	0.000	0.115	0.000	0.000	0.000	0.000	0.000	0.000	E107G (GAG→GGG)	*Synpcc7942_2610* ←	uroporphyrinogen III C methyltransferase

Position	Mutation	SynLL_17	SynLL_18	SynLL_19	SynLL_20	SynLL_21	SynLL_22	SynLL_23	SynLL_24	Annotation	Gene	Description
30,885	A→G	0.000	0.000	0.137	0.000	0.000	0.000	0.000	0.000	D16G (GAT→GGT)	*Synpcc7942_0030* →	dethiobiotin synthase
51,345	G→T	0.000	0.000	0.000	0.000	0.000	0.134	0.000	0.000	E426* (GAA→TAA)	*Synpcc7942_0050* →	conserved hypothetical protein
52,960	A→G	0.000	0.000	0.000	0.000	0.076	0.000	0.000	0.000	D516D (GAT→GAC)	*Synpcc7942_0051* ←	TPR repeat
92,978	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Q121R (CAG→CGG)	*Synpcc7942_0095* ←	two component transcriptional regulator, winged helix family
93,288	C→A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	V18F (GTC→TTC)	*Synpcc7942_0095* ←	conserved hypothetical protein
132,842	T→C	0.000	0.000	0.183	0.000	0.000	0.000	0.000	0.000	D310G (GAC→GGC)	*Synpcc7942_0132* ←	conserved hypothetical protein
199,903	T→C	0.000	0.000	0.000	0.190	0.000	0.000	0.000	0.000	E176G (GAG→GGG)	*Synpcc7942_0198* ←	type 2 NADH dehydrogenase
338,303	A→G	0.000	0.126	0.000	0.000	0.000	0.000	0.000	0.000	L50P (CTC→CCC)	*Synpcc7942_0344* ←	long chain fatty acid CoA ligase
354,748	T→G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Y73D (TAC→GAC)	*Synpcc7942_0361* →	sodium dependent bicarbonate transporter
382,629	T→C	0.000	0.000	0.000	0.102	0.000	0.000	0.000	0.000	S276G (AGC→GGC)	*Synpcc7942_0390* ←	Chromate transporter
427,308	G→C	0.000	0.000	0.000	0.061	0.000	0.000	0.000	0.000	intergenic (+158/ 155)	*Synpcc7942_0436* → / → *Synpcc7942_0437*	hypothetical protein/putative glutathione peroxidase
589,398	C→G	0.099	0.000	0.000	0.000	0.000	0.000	0.000	0.000	L221L (CTG→CTC)	*Synpcc7942_0600* ←	conserved hypothetical protein
684,439	T→C	0.000	0.000	0.146	0.000	0.000	0.000	0.000	0.000	E149G (GAG→GGG)	*Synpcc7942_0689* ←	hypothetical protein
738,978	T→C	0.000	0.000	0.000	0.130	0.000	0.000	0.000	0.000	S274P (TCT→CCT)	*Synpcc7942_0744* →	conserved hypothetical protein
803,550	A→T	0.000	0.000	0.000	0.175	0.000	0.000	0.000	0.000	I160F (ATT→TTT)	*Synpcc7942_0809* →	conserved hypothetical protein
826,882	A→G	0.000	0.000	0.000	0.000	0.000	0.107	0.000	0.000	intergenic (877/ 230)	*Synpcc7942_0831* ← / → *Synpcc7942_0833*	conserved hypothetical protein/conserved hypothetical protein
924,962	T→C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	L295P (CTG→CCG)	*Synpcc7942_0918* →	conserved hypothetical protein
985,783	C→T	0.000	0.078	0.000	0.000	0.000	0.000	0.000	0.000	A76A (GCG→GCA)	*Synpcc7942_0977* ←	Glucokinase regulatory like protein
1,027,017	A→G	0.000	0.000	0.000	0.000	0.000	0.000	0.226	0.000	S467P (TCA→CCA)	*Synpcc7942_1014* ←	CheA signal transduction histidine kinase
1,197,302	G→T	0.000	0.107	0.000	0.000	0.000	0.000	0.000	0.000	intergenic (+810/ 115)	*Synpcc7942_1163* → / → *Synpcc7942_1164*	two component transcriptional regulator, winged helix family
1,214,163	T→C	0.000	0.000	0.137	0.000	0.000	0.000	0.000	0.000	P228P (CCT→CCC)	*Synpcc7942_1183* →	conserved hypothetical protein
1,412,437	C→A	0.000	0.000	0.000	0.086	0.000	0.000	0.000	0.000	Q960K (CAA→AAA)	*Synpcc7942_1372* →	methionine synthase (B12 dependent)
1,443,793	G→A	0.000	0.000	0.000	0.000	0.000	0.185	0.000	0.000	R83H (CGC→CAC)	*Synpcc7942_1394* →	PDZ/DHR/GLGF
1,526,404	C→T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	G184D (GGC→GAC)	*Synpcc7942_1475* ←	two component transcriptional regulator, winged helix family
1,532,790	T→C	0.000	0.000	0.000	0.170	0.000	0.000	0.000	0.000	T62T (ACT→ACC)	*Synpcc7942_1482* →	conserved hypothetical protein
1,727,635	T→C	0.000	0.000	0.000	0.000	0.101	0.000	0.000	0.000	A149A (GCA→GCG)	*Synpcc7942_1656* ←	catalase/peroxidase HPI
1,867,792	G→T	0.000	0.000	0.079	0.000	0.000	0.000	0.000	0.000	intergenic (+416/ 72)	*Synpcc7942_1797* → / → *Synpcc7942_1798*	conserved hypothetical protein/conserved hypothetical protein
1,964,447	+GATGGTAATAA	0.000	0.000	0.000	0.000	0.000	0.090	0.000	0.000	coding (36/561 nt)	*Synpcc7942_1891* →	conserved hypothetical protein
1,964,485	G→A	0.090	0.000	0.000	0.000	0.000	0.000	0.000	0.000	G25D (GGT→GAT)	*Synpcc7942_1891* →	conserved hypothetical protein
1,964,557: 1	+T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	coding (146/561 nt)	*Synpcc7942_1891* →	conserved hypothetical protein
1,964,744: 1	+T	0.000	0.000	0.000	0.082	0.000	0.000	0.000	0.000	coding (333/561 nt)	*Synpcc7942_1891* →	
1,964,806	G→T	0.616	0.545	0.443	0.479	0.587	0.376	0.674	0.690	R132L (CGC→CTC)	*Synpcc7942_1891* →	long chain fatty acid CoA ligase
1,964,893	T→G	0.333	0.459	0.277	0.385	0.229	0.306	0.000	0.173	L161R (CTG→CGG)	*Synpcc7942_1891* →	conserved hypothetical protein/conserved hypothetical protein
2,158,496	G→A	0.235	0.421	0.393	0.320	0.343	0.380	0.325	0.140	L153L (TTG→TTA)	*Synpcc7942_2080* →	conserved hypothetical protein
2,179,976	T→A	0.000	0.000	0.000	0.000	0.000	0.000	0.124	0.000	E139V (GAG→GTG)	*Synpcc7942_2097* ←	conserved hypothetical protein
2,208,804	A→T	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	D80V (GAT→GTT)	*Synpcc7942_2130* →	Fructose 6 phosphate phosphoketolase
2,370,611	C→T	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	P24S (CCA→TCA)	*Synpcc7942_2301* →	hypothetical protein
2,392,649	A→G	0.000	0.000	0.000	0.000	0.000	0.144	0.000	0.000	L149S (TTG→TCG)	*Synpcc7942_2325* ←	PBS lyase HEAT like repeat
2,662,727	Δ7 bp	0.903	0.894	0.788	0.900	0.889	0.857	1.000	0.936	coding (360 366/924 nt)	*Synpcc7942_2577* ←	conserved hypothetical protein
2,670,870	G→T	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	S175* (TCA→TAA)	*Synpcc7942_2587* ←	conserved hypothetical protein

APPENDIX H: RAW UPLC DATA EXPERIMENT 3

Data for 3-14-2020

	White light						Green light						Orange light					
	Acetone extract	Rxn Product	Pellet	Sum	Percent acetone	Percent Rxn prod	Acetone extract	Rxn Product	Pellet	Sum	Percent acetone	Percent Rxn prod	Acetone extract	Rxn Product	Pellet	Sum	Percent acetone	Percent Rxn prod
Sample 1	1.09	0.30	5.92	7.31	14.91	4.10	0.98	0.27	4.47	5.72	17.13	4.72	0.94	0.40	3.30	4.64	20.26	8.62
Sample 2	1.21	0.35	7.29	8.85	13.67	3.95	0.98	0.27	3.40	4.65	21.08	5.81	0.69	0.25	3.87	4.81	14.35	5.20
Sample 3	0.84	0.34	4.33	5.51	15.25	6.17	1.21	0.35	5.05	6.61	18.31	5.30	0.69	0.26	3.73	4.68	14.74	5.56
Average	1.05	0.33	5.85	7.22	14.61	4.74	1.06	0.30	4.31	5.66	18.84	5.27	0.77	0.30	3.63	4.71	16.45	6.46
SD	0.19	0.03	1.48	1.67	0.83	1.24	0.13	0.05	0.84	0.98	2.02	0.54	0.14	0.08	0.30	0.09	3.31	1.88
RSD	18.04	8.02	25.34	23.14	5.67	26.11	12.57	15.57	19.44	17.34	10.75	10.30	18.66	27.65	8.18	1.89	20.09	29.14

Suspect value. Could not read tare well.

Component Name	Origin Index	Equation													
Phycocyanobillin-I	Ignore	Y = -4.44737e+006+1.605e+007*X R^2 = 0.9938													
Filename	Exp Amt	Calc Amt	Average concentration (n=3)	Standard deviation	RSD	Compound concentration before dilution	Extract weight	Total sample weight	Extract concentration (ug/mL)	Percent of extract	Percent of sample	Average percent of sample	Standard deviation (n=3)	RSD (n=3)	
G1-RP-1x 01	NA	1.203	1.183	0.025	0.022	5.916	0.27	5.72	1350	0.438	0.021				
G1-RP-1x_02	NA	1.154	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G1-RP-1x_03	NA	1.193	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G2-RP-1x_01	NA	0.719	0.718	0.002	0.002	3.591	0.27	4.65	1350	0.266	0.015	0.051	0.056	111.462	Green light
G2-RP-1x_02	NA	0.720	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G2-RP-1x_03	NA	0.716	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G3-RP-1x_01	NA	7.629	7.641	0.127	0.017	38.207	0.35	6.61	1750	2.183	0.116				
G3-RP-1x_02	NA	7.775	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G3-RP-1x 03	NA	7.521	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O1-RP-1x 01	NA	0.316	0.300	0.015	0.049	1.501	0.4	4.64	2000	0.075	0.006				
O1-RP-1x_02	NA	0.287	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O1-RP-1x_03	NA	0.298	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O2-RP-1x_01	NA	0.446	0.446	0.001	0.001	2.230	0.25	4.81	1250	0.178	0.009	0.007	0.002	24.805	Orange light
O2-RP-1x_02	NA	0.446	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O2-RP-1x_03	NA	0.447	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O3-RP-1x 01	NA	0.278	0.278	0.000	0.001	1.388	0.26	4.68	1300	0.107	0.006				
O3-RP-1x_02	NA	0.278	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O3-RP-1x 03	NA	0.277	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W1-RP-1x 03	NA	1.123	1.130	0.029	0.026	5.651	0.3	7.31	1500	0.377	0.015				
W1_RP-AE_01	NA	1.163	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W1_RP-AE_02	NA	1.105	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W2-RP-1x_01	NA	1.089	1.086	0.015	0.014	5.429	0.35	8.85	1750	0.310	0.012	0.011	0.005	42.223	White light
W2-RP-1x_02	NA	1.098	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W2-RP-1x_03	NA	1.070	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W3-RP-1x 01	NA	0.337	0.336	0.001	0.003	1.680	0.34	5.51	1700	0.099	0.006				
W3-RP-1x 02	NA	0.335	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W3-RP-1x 03	NA	0.335	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				

Component Name	Origin Index	Equation													
Chlorophyll-A	Ignore	Y = 1.27807e+006+4.13886e+007*X R^2 = 0.9797													
Filename	Exp Amt	Calc Amt	Average concentration (n=3)	Standard deviation	RSD	Compound concentration before dilution	Extract we	Total sam	Extract co	Percent of	Percent of	Average p	Standard	(RSD (n=3)	
GI-AE_1200x_01	NA	0.848	0.866	0.017	0.020	1039.338	0.98	5.72	4900	21.211	3.634				
GI-AE_1200x_02	NA	0.882	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
GI-AE_1200x_03	NA	0.869	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G2-AE_1200x_01	NA	0.576	0.562	0.024	0.044	674.352	0.98	4.65	4900	13.762	2.900	3.637	0.738	20.291	Green light
G2-AE_1200x_02	NA	0.576	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G2-AE_1200x_03	NA	0.534	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G3-AE_1200x_01	NA	1.187	1.205	0.029	0.024	1446.398	1.21	6.61	6050	23.907	4.376				
G3-AE_1200x_02	NA	1.190	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G3-AE_1200x_03	NA	1.239	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O1-AE_1200x_01	NA	0.567	0.581	0.034	0.059	697.790	0.94	4.64	4700	14.847	3.008				
O1-AE_1200x_02	NA	0.557	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O1-AE_1200x_03	NA	0.621	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O2-AE_1200x_01	NA	0.228	0.256	0.025	0.099	307.386	0.69	4.81	3450	8.910	1.278	1.979	0.910	45.986	Orange light
O2-AE_1200x_02	NA	0.277	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O2-AE_1200x_03	NA	0.263	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O3-AE_1200x_01	NA	0.282	0.322	0.035	0.110	386.483	0.69	4.68	3450	11.202	1.652				
O3-AE_1200x_02	NA	0.333	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O3-AE_1200x_03	NA	0.351	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W1-AE_1200x_01	NA	0.892	0.929	0.034	0.036	1114.248	1.09	7.31	5450	20.445	3.049				
W1-AE_1200x_02	NA	0.935	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W1-AE_1200x_03	NA	0.959	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W2-AE_1200x_01	NA	1.077	1.100	0.038	0.034	1320.058	1.21	8.85	6050	21.819	2.983	3.083	0.120	3.901	White light
W2-AE_1200x_02	NA	1.143	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W2-AE_1200x_03	NA	1.080	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W3-AE_1200x_01	NA	0.738	0.738	0.006	0.008	886.095	0.84	5.51	4200	21.098	3.216				
W3-AE_1200x_02	NA	0.744	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W3-AE_1200x_03	NA	0.733	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				

Component Name	Origin Index	Equation													
beta-carotene	Ignore	Y = -196622+864571*X R^2 = 0.9218													
Filename	Exp Amt	Calc Amt	Average c	Standard (	RSD	Compoun	Extract we	Total sam	Extract co	Percent of	Percent of	Average p	Standard (	RSD (n=3)	
G1-AE_1200x_01	NA	0.276	0.279	0.003	0.010	334.486	0.98	5.72	4900	6.826	1.170				
G1-AE_1200x_02	NA	0.279	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G1-AE_1200x_03	NA	0.281	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G2-AE_1200x_01	NA	0.265	0.261	0.003	0.012	313.233	0.98	4.65	4900	6.393	1.347	1.215	0.116	9.558	Green ligh
G2-AE_1200x_02	NA	0.260	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G2-AE_1200x_03	NA	0.259	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G3-AE_1200x_01	NA	0.277	0.311	0.030	0.095	373.079	1.21	6.61	6050	6.167	1.129				
G3-AE_1200x_02	NA	0.327	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
G3-AE_1200x_03	NA	0.328	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O1-AE_1200x_01	NA	0.236	0.239	0.005	0.022	286.454	0.94	4.64	4700	6.095	1.235				
O1-AE_1200x_02	NA	0.235	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O1-AE_1200x_03	NA	0.245	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O2-AE_1200x_01	NA	0.228	0.228	0.000	0.002	273.513	0.69	4.81	3450	7.928	1.137	1.181	0.050	4.195	Orange lig
O2-AE_1200x_02	NA	0.228	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O2-AE_1200x_03	NA	0.228	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O3-AE_1200x_01	NA	0.228	0.228	0.000	0.002	273.900	0.69	4.68	3450	7.939	1.171				
O3-AE_1200x_02	NA	0.228	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
O3-AE_1200x_03	NA	0.229	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W1-AE_1200x_01	NA	0.272	0.315	0.038	0.120	378.293	1.09	7.31	5450	6.941	1.035				
W1-AE_1200x_02	NA	0.329	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W1-AE_1200x_03	NA	0.344	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W2-AE_1200x_01	NA	0.779	0.818	0.036	0.044	981.432	1.21	8.85	6050	16.222	2.218	1.518	0.621	40.910	White ligh
W2-AE_1200x_02	NA	0.823	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W2-AE_1200x_03	NA	0.851	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W3-AE_1200x_01	NA	0.319	0.298	0.018	0.059	358.057	0.84	5.51	4200	8.525	1.300				
W3-AE_1200x_02	NA	0.287	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				
W3-AE_1200x_03	NA	0.289	0.000	0.000	0.000	0.000	0	0	0	0.000	0.000				