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Analysis of Growth Responses in *Saccharina latissima* Gametophytes in Different Nutrient Solutions and Profiling of Peroxidases in Sporophytes under Light Stress.

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Abstract:

Saccharina latissima a brown sugar kelp is a highly nutritional seaweed, grows well in the Norwegian climate and can be used as food and feed. Different research studies were conducted to find the optimal nutritional content and environmental conditions that promoted the growth. The current study is designed with two important but different aims: the first study is designed to test the impact of three different nutrient solutions (i.e. ½ PES (Provasoli Enriched seawater), F/2 (Guillard Marine Enrichment medium), and ES(Enriched Seawater) based on nutritional value) on the growth of *S. latissima* gametophytes under red light stress conditions; the second study is done to understand the regulation of gene expression in response to light intensity stress stimuli in *S. latissima* sporophyte stage by taking a holistic approach to uncover the intricate relationship between peroxidase genes and environmental stimuli in *S. latissima*.

In first study, 1/2 PES and F/2 came out to be nutrient rich that promoted the growth of *S. latissima* whereas ES showed minimal impact on growth, regarded as nutrient deficient. These results imply that nutrient rich solutions can be used to promote the growth of kelp.

In second study, different peroxidase genes showed different expression under three different red-light intensities. Most of the heme and non-heme peroxidase genes e.g. *Apx01, NAnprx Cp03*, showed upregulation under medium and maximum red intensity level 1000 µmol m^{-2} $s^{-1}$and 250 µmol m^{-2} s^{-1} respectively. While some peroxidase genes *Ccp0,Cp04 and Pxd04* showed downregulation at medium and maximum red-light intensity 100 µmol m^{-2} s^{-1} and 250 µmol m^{-2} s^{-1} respectively, suggested a fine-tuned cellular response, indicating an adaptive mechanism for protection against light stress in the sporophyte.

Further studies are recommended to analyze the underling mechanism in short duration experiments. Overall, our research shows growth and gene regulation is highly influenced by external factors like light and temperature.

Table of content

Introduction

Background

Sugar kelp (*Saccharina latissima*), a *Laminariales* species, is extensively found in the Northern Hemisphere and has been a subject of study since the 60s due to its ecological significance along western temperate coasts. Recently, research interest has been urged, fueled by concerns about the adverse effects of human-induced environmental changes and growing commercial interest in cultivating the species. The biomass of sugar kelp holds industrial applications (biofuel production, biodegradable plastics, fertilizers and food additives etc.), further motivating investigations into its physiology and ecology.

Literature review

Kelps are brown macroalgae (Phaeophyceae) defined as representatives of the order *Laminariales* exclusively. The genera *Alaria, Laminaria,* and *Saccharina* are primarily represented among kelps in the Northern Hemisphere (Bolton, 2010; Filbee-Dexter et al., 2019). A taxonomic reorganization based on genetic evidence was proposed in 2006, reassigning the previously *Laminaria saccharina* to *Saccharina latissima*, the now accepted species name (Lane et al., 2006).

This species typically grows on shallow rocky coasts along the Atlantic, Pacific, and Indian Oceans in the upper subtidal to depths of 15-30 m, attaching to hard rock as well as boulders and cobbles with a branching claw-like holdfast (Nora et al., 2023). Long, ribbon-like blades with an average length of 1 to 4 meters are among its morphological characteristics (Pereira et al., 2016). The shape of the *S. latissima* sporophyte varies widely depending on exposure and environmental conditions (Rupe´rez, 2002).

S. latissima is commonly referred to as 'sugar kelp', because of the delicious white powder (mannitol) that stays on the seaweed's surface as it dries. According to a sensory investigation, with the combination of salts with sweet flavor consumers ranked *S. latissima* as versatile flavor among the three species investigated, including *Laminaria digitata* and *Alaria esculenta* (Chapman et al., 2015).

Although the number of studies on *S. latissima*'s environmental adaptation is significant, it's adaptation differs depending on the factors and geographic location. The content and concentration of biological substances such as pigments, carbohydrates, antioxidants, lipids, fatty acids, and proteins in seaweeds changes depending on environmental conditions (Amsler, 2008; Bartsch et al., 2008).

Extensive studies have been performed about the life cycle and development of *S latissima* under different temperature levels, and light levels (Lüning & Dring, 1975). In addition, a thorough

investigation of the distribution and ecological significance of *S. latissima* in the Arctic was carried out by Bartsch et al., (2008), about how the species has evolved to cope with changing environmental conditions. The ecological relationships between sugar kelp and other marine organisms in the habitat were investigated by Christie et al., (2019). Their investigation focused on the intricate interactions between other algae, herbivores, and sugar kelp. For the protection and management of coastal ecosystems, where Sugar kelp plays an important role, it is imperative to comprehend these ecological dynamics.

Recent study looked at the role sugar kelp plays in reducing ocean acidification by absorbing more CO_2 and fostering pH values that are good for some marine species (Nielsen et al., 2016). Results highlight how crucial sugar kelp is to preserve the viability of coastal ecosystem, because its multifaceted ecological contributions e.g; biodiversity support, nutrient regulation, erosion control, oxygen production,

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Rupe´rez et al., (2002) promoted the use of sugar kelp in the food business by examining its nutritive and sensory properties. These investigations have paved the way for more investigation and practical uses of sugar kelp. The nutritional and gastronomic possibilities of sugar kelp and other seaweeds were investigated in a study by Kim et al., (2017) . They focused on sugar kelp's function as a sustainable and nutrient-rich food source as they studied its nutritional makeup and culinary uses. The study promoted the use of sugar kelp in contemporary cuisine by highlighting its culinary variety and health benefits.

Life cycle strategy

The life cycle of *S. latissima* is heteromorphic (haploid/diploid) (Coelho et al. 2019). It alternates between large, sporophytes and microscopic gametophytes (Fig. 1).

Sporophyte Phase:

Sessile macroscopic sporophytes (2n) can grow up to 4 meters in length (White and Marshall 2007) and have a wide range of morphological appearances (Nora et al., 2023).

Reproduction of Sporophytes:

- On the sporophyte, sporangia, which are specialized structures, appear. Spore mother cells can be found in these sporangia.
- To produce haploid spores, sporangia's spore mother cells go through meiosis.
- The release of these spores into the water causes them to disperse.

Gametophyte Phase:

- **Spore Germination**: Spores germinate and grow into microscopic, filamentous gametophytes when they land on and connect to a suitable substrate (Goecke et al., 2022; Lüning & Neushul, 1978).
- **Growth of gametophytes**: Gametophytes develop and produce male and female gametangia over time.

Reproduction of Gametophytes:

- Gametangia are specialized structures that hold gametes that are produced by male and female gametophytes.
- **Release of Gametes**: While female gametangia release eggs, male gametangia discharge sperm cells. In the water, fertilization takes place when male gametangia's sperm swim to reach and fertilize female gametangia's eggs, creating a zygote.

Development of Zygotes:

The initiation of the diploid phase begins with an elongated zygote when the male gamete is chemically attracted by female gametophyte egg. After only a few months, the developing embryo will mature into an impressive (>3 m) sporophyte (Theodorou et al., 2021).

Gametes and gametophytes have sexually dimorphic characteristics, and sex is expressed during the haploid stage. It is possible to distinguish and separate the sexes in the lab because male gametophyte cells are smaller and tend to form filaments with more cells than female gametophyte cells and nuclei, which are larger and rounder (Figure: 01), (Goecke et al., 2022)

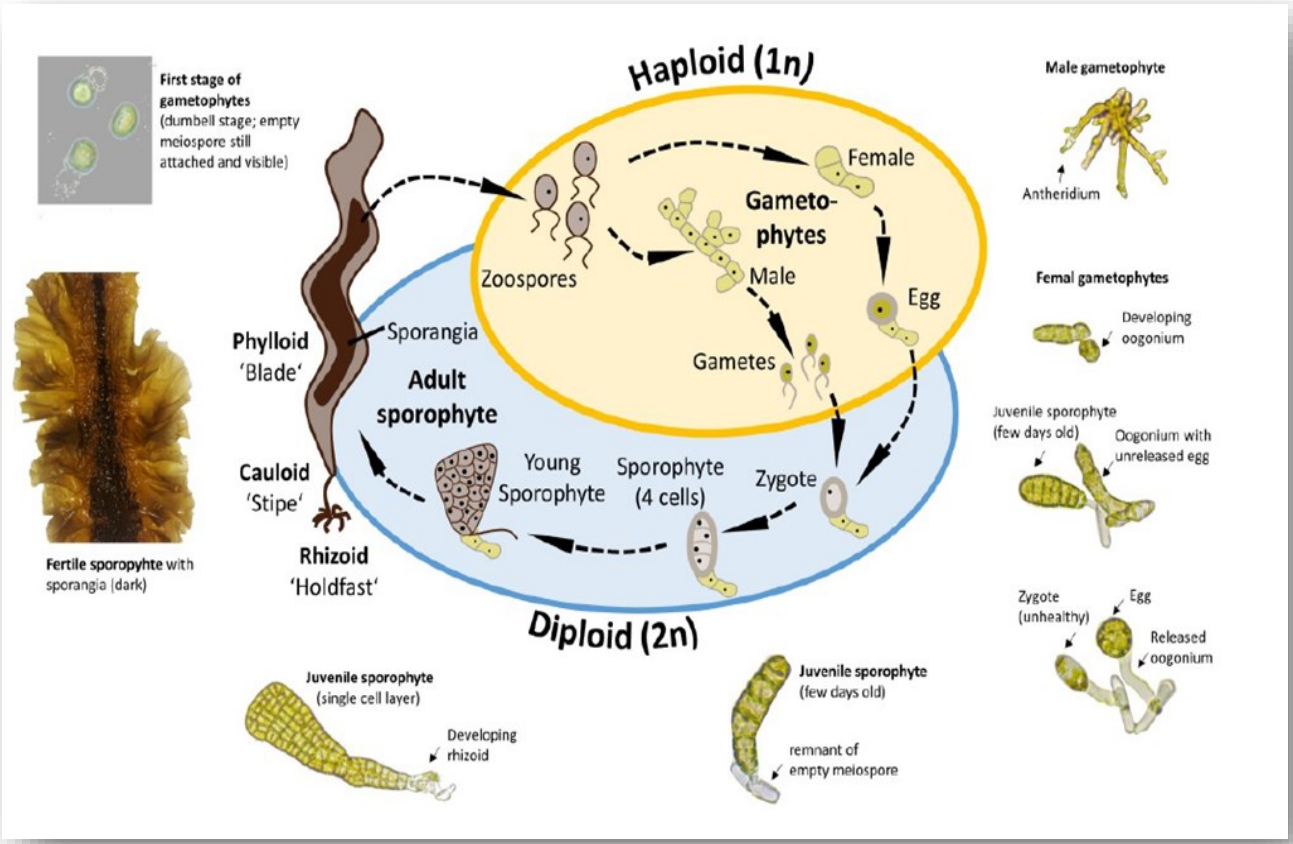

Figure 1 ***Saccharina latissima's*** **life cycle.** *S. latissima* has two distinct phases in its life cycle: a haploid (yellow) phase and a diploid (blue) phase. Zoospores are released by adult sporophytes (2n) and develop into male or female gametophytes (1n). Male and female gametophytes release gametes and eggs, respectively (1n). A zygote (2n) formed from the fusion of an egg and gametes develop into sporophytes (2n) **after** ***Diehl et al., (2023)***.

Sugar kelp's ecological Importance

S. latissima like the other kelp species provides valuable ecosystem services with large ecological and economic value(Eger et al., 2023).

1. Improvement of Water Quality and Nutrient Uptake

Because of its capacity to absorb nutrients, sugar kelp is an essential component of marine ecosystems, it removes extra nutrients from the water, reducing the negative impacts of eutrophication (Kim et al., 2017).

2. Environment and Biodiversity

S. latissima provides refuge and protection for a variety of marine creatures. The varied biodiversity that these kelp forests support makes them crucial fish species nesting grounds (Wernberg & Filbee-Dexter, 2019). This has ramifications for both commercial fishing and the marine ecology.

3. Carbon Sequestration

Carbon dioxide (CO_2) sequestration is one of sugar kelp's important ecological functions. It reduces the consequences of ocean acidification and global climate change because it is a photosynthetic organism that absorbs CO_2 as it grows (Doney et al., 2020).

Applications of Sugar kelp in Industry

1. Culinary and Food Uses

The ability of sugar kelp to be used as a component in a variety of cuisines has been quickly acknowledged by the culinary world. Due to its umami-rich flavor, it is used in salads, soups, and as a flavor enhancer. Sugar kelp is a nutritious complement to meals because it also includes vital nutrients such as iodine, vitamin (A, C and K), minerals (iron , calcium and magnesium) Rupe´rez et al., (2002).

2. Production of Bioenergy

Biofuels and bioplastics can be produced using sugar kelp. A sustained source of energy is made possible by its high carbohydrate content, primarily alginate, which may be turned into bioethanol Mata et al., (2010).

3. Medicinal products and biotechnology

The potential of *S. latissima* for a variety of uses has been investigated by the pharmaceutical sector. Sugar kelp is a useful resource for medical and biotechnological uses since compounds isolated from it have demonstrated promising antibacterial and antioxidant effects. For many years, kelp has been utilized in Europe to treat iodine-deficiency disorders Eger et al., (2023)

4. Fertilizers and Agriculture

Agriculture uses *S. latissima* kelp extracts as organic fertilizers. They promote plant growth, strengthen the soil, and offer necessary micronutrients Zheng et al., (2016).

Macroalgae industry and economic values of S. latissima:

S. latissima has a number of benefits over other viable seaweeds when it comes to selective breeding, including the following:

1. Complete control over its life cycle;

2. the ability to isolate and grow male and female gametophytes through vegetative reproduction;
3. The ability to choose and breed throughout both the gametophytic and sporophytic phases Visch et al., (2019)

Macroalgae has long been regarded as a valuable food source, particularly in East Asian countries. In Norway, algae have traditionally been used as feed and fertilizer. Today, macroalgae are used for food, feed, dietary supplements, medicines, alginate manufacturing, biofuel, and other purposes Goecke et al., (2020). Over the previous 20 years, overall macroalgae output has increased significantly to satisfy demand. Globally, 31.2 million tons of fresh weight were produced in 2016, with output likely to rise further Goecke et al., (2022).

In order to ensure the health of macroalgae-dependent ecosystems, future macroalgae extraction must be based on cultivation. Norway harvested around 150,000 tons of macroalgae in 2015 (mainly from wild reaping), accounting for 65% of the overall European supply (EU 2018). *S. latissima* is a prevalent species for cultivation that has been successfully grown throughout Europe. Minerals, phenolic compounds, carbohydrates, and polyunsaturated fatty acids are among the beneficial nutritional ingredients found in S. *latissima*.

Stress factors play a pivotal role in influencing the overall health and resilience of *S. latissima*. Understanding how these stressors impact its growth and survival is integral to comprehending the intricate dynamics of this marine species in its natural habitat and in industrial applications.

Stress Factors:

1. Light stress:

   Seaweeds are photosynthetic organisms; their survival depends on the availability of light. The effects of irradiance on *S. latissima* have already been well investigated over many years Bartsch et al., (2008). In *S. latissima*, variations in susceptibility can be observed in a number of biochemical and physiological processes due to variations in both extremely high and low levels. More recent research showed that decreased irradiance still promotes biofouling Forbord et al., (2020) while adversely affecting sporophytes' in situ growth performance without compromising photosynthetic performance Spurkland & Iken et al., (2011).

2. Temperature Stress:

   Temperature has a substantial influence on the composition and biogeographical distribution patterns of macroalgal communities (Heinrich et al., 2012; Lüning, 1984). Helgoland sporophytes grew best around 10 to 15°C (Bolton & lüning, 1982). However, they could withstand a wide temperature range of 0 to 23°C for shorter periods of time, with mortality rates rapidly rising above 20°C (Lüning, 1980).

According to Williams SL, (1989), *S. latissima* gametophytes showed a wider range of temperature tolerance, withstanding temperatures as low as -1.5°C and as high as 23–25°C. Additionally, variations in temperature sensitivity were discovered between male and female gametophytes (Monteiro et al., 2019) as well as between field sporophytes and laboratory cultures (Heinrich S, 2016). Significant variations in *S. latissima*'s physiological state were noted in Norway; for example, a hotter year showed greater erosion and less development than a colder year (Armitage CS, 2017).

3. Stress due to salinity:

   There is little known about the relationship of salinity and other variables in *S. latissima*, with only salinity temperature being studied thus far. Recent research has demonstrated that hypo-salinity, when combined with temperature variation, can be extremely stressful for *S. latissima*. Low salinity combined with high summer temperatures in the Baltic Sea reduces S. *latissima* productivity due to significant physiological stress in cultivated seaweed Nielsen et al., (2014).

4. Stress due to nutrients:

   Nitrogen (N) is the primary resource that limits macroalgal productivity, whereas phosphorus (P) is also a macronutrient that is necessary for photosynthesis and growth (Roleda & Hurd, 2019). It is well recognized that *S. latissima*'s physiological status is negatively impacted by nutrient deficiency; this can lead to reduced growth rate and photosynthetic performance, for example (Roleda & Hurd, 2019; Williams & Herbert, 1989)

5. Ocean acidification:

   The continuous drop in seawater pH and changes in carbonate chemistry brought on by the significant marine intake of CO_2 since the Industrial Revolution are referred to as ocean acidification (OA) Doney et al., (2020). Research on how OA affects *S. latissima* has mostly concentrated on three areas: growth, photophysiology, and biochemistry. According to the length of the experiment and the applied partial pressure of CO_2 (pCO_2), OA has been shown to increase (Gordillo FJL, 2015), not affect or even decrease the growth rates of *S. latissima*.

6. Physical Stress:

   Due to numerous mechanical forces, sugar kelp may get physically stressed. The kelp may get physically harmed or dislodged due to wave action, sedimentation, and animal grazing. The resilience and structural integrity of the kelp in its ecosystem may be impacted by these physical disturbances (Bennett et al., 2015).

Responses to Stress at the Sporophyte Phase:

Sporophytes may show several adaptation strategies in response to stressors. For instance, to battle oxidative stress brought on by intense light or high temperatures, they might activate genes involved in antioxidant defense. To adapt to nutritional shortages, sporophytes may potentially alter the expression of genes involved in nutrient intake and utilization. The goal of the transcriptome reactions is to keep cells in a state of homeostasis and lessen the negative effects of stress on development and reproduction Dittami & Tonon, (2020).

Responses to stress at the gametophyte phase:

Gametophytes, which are in the reproductive phase, may give priority to reproductive functions when resources are limited. They may control gene expression related to gamete formation and fertilization in response to environmental stresses. The success of sexual reproduction in the life cycle may be impacted by the transcriptome alterations in gametophytes, which may also involve the overexpression of genes involved in stress signaling and defense mechanisms (Dittami & Tonon, 2020).

The interrelationships between the impacts of stress events on the transcriptome and the subsequent responses in sugar kelp sporophytes and gametophytes are a component of the species' adaptation to changing maritime environments.

RNA Sequencing:

The expression “RNA-sequencing” or “RNA-seq” was first coined in 2008 (Nagalakshmi et al., 2008) and has since then revolutionized transcriptomics. RNA-seq is defined by the reverse transcription of extracted RNA into double-stranded complementary DNA (ds-cDNA), which is subsequently sequenced using various sequencer machines (Weber, 2015). The technology has a number of advantages over approaches such as Sanger sequencing and microarray-based sequencing: first, unlike microarray techniques, RNA-seq does not rely on previously identified sequences and can therefore be used to find new sequences; additionally, compared to Sanger and microarray-based methods, RNA-seq offers better coverage and resolution (Kukurba & Montgomery, 2015). Even now, RNA-seq technology is continually evolving, with new advancements being made such as single cell RNA sequencing(sc-RNAseq), spatial transcriptomics, long read sequencing.

To link sequenced reads to previously identified genes and gene products in annotated genomes, several techniques are available. Blastx is the computational procedure for aligning transcript sequences to protein sequences that are known already. The newly released Diamond program promises comparable sensitivity but faster execution than earlier blastx softwares (Buchfink et al., 2021). Since its founding as a project to compile genes associated with essential biological functions into a single database (Ashburner et al., 2000), the Gene Ontology Consortium (GOC) has evolved into a go-to

source for transcriptome studies pertaining to the annotation of genes for biological functions (Carbon et al., 2021).

As of right now, the genomic sequences of two brown algae species—*S. japonica* (Ye et al., 2015) and *Ectocarpus siliculosus* (Cock et al., 2010) are complete. Transcriptome projects on *S. latissima* must rely on these genomes until the entire genome is sequenced.

Transcriptomic analysis on sporophytes under light stress conditions:

Sporophytes can experience stress from changes in light quality or intensity, which can have an impact on their growth, physiology, and general health.

1. Upregulation of Light-harvesting Complexes (LHCs): Sporophytes may upregulate the expression of genes associated with LHCs in order to improve their capacity to absorb light for photosynthesis when exposed to low light levels (Roeder, 2005).
2. Photoprotective systems: Sporophytes may activate photoprotective systems in high-light environments. The overexpression of genes linked to non-photochemical quenching (NPQ) activities, which release surplus energy as heat to protect the photosynthetic apparatus, is a sign of this (Johansson & Nylander, 1996).
3. Stress-response Proteins: the generation of reactive oxygen species (ROS) by high light intensities can lead to oxidative stress. To battle oxidative stress, the transcriptome may show an increase in the expression of antioxidant enzymes including superoxide dismutase (SOD) and catalase (Collén & Davison, 1999).
4. Responses involving chloroplasts: genes involved in chloroplast biosynthesis, maintenance, and repair may express themselves differently in response to light stress. This is a result of the sporophyte's efforts to keep its photosynthetic system in good working order (Roeder et al., 2005).
5. Regulation of the Cell Cycle and Growth: according to Collén and Davison (1999), excessive or insufficient light can impede growth by altering the expression of genes involved in DNA replication, repair, and cell cycle.
6. Changes in Metabolism: Different metabolic pathways can be impacted by light stress. An increase in the expression of genes involved in glucose metabolism, for instance, could provide more energy and metabolites to deal with stress (Roeder et al., 2005).

Peroxidases:

Peroxidases are a class of enzymes that are essential for many biological activities, especially the detoxification of reactive oxygen species (ROS) and the control of oxidative stress. These enzymes

make it easier for hydrogen peroxide (H_2O_2) to break down into water and oxygen, which is a critical step in preventing oxidative cell damage(Mittler, 2002).

Peroxidases are heme or no heme-containing enzymes, these are the two primary subtypes of peroxidases. These classifications are based on whether an iron-containing molecule called heme is present or absent in the structure of the enzyme that uses hydrogen peroxide as the electron acceptor to catalyze the oxidation of a variety of substrates. They contribute to the preservation of cellular redox equilibrium and general cell health as important elements of antioxidant defense mechanisms in both plants and mammals (Davies & Hawkins, 2020).

Heme Peroxidases:

Heme groups act as a prosthetic group in the active site of the peroxidases known as heme peroxidases. The binding of substrates and catalysis occurs at the heme group. Heme peroxidases have further different classes and have different roles, like (Table:1) (Savelli et al., 2019)

Class	Acronym	Role
▼**Non-Animal peroxidase**		
Ascorbate peroxidase	*APx*	Chloroplastic organisms contain APx. They have a significant selectivity for the electron donor ascorbate.
Catalase peroxidase	*CP*	CP, HPI, and KatG are primarily found in prokaryotes, with minor amounts found in fungi and protists. They are fusion proteins with dual catalytic activity, operating as both a catalase and a peroxidase.
Cytochrome C peroxidase	*CcP*	CcP serve an important role in recycling H_2O_2 produced during aerobic respiration from cytochrome c oxidation in the mitochondrial intermembranous region. Non-mitochondrial organisms do not have CcP.
Other non-animal peroxidase	*NAnPrx*	It is a homodimeric peroxidase that is expressed constitutively and is present in the cytosol, peroxisomes, and mitochondria.
▼**Peroxidase-Cyclooxygenase superfamily**		

Peroxidasin	*Pxd*	Members of this superfamily have a covalent bond with the apoprotein through their prosthetic heme group. Many of them are fusion proteins with non-peroxidase domains

Table 1 Heme peroxidase with different classes and their roles.

Heme Peroxidases' Response to Light Stress:

Through a number of mechanisms, notably those connected to the production of reactive oxygen species (ROS) during photosynthesis, light stress can influence how peroxidases react. A surplus of energy produced by high light levels may result in the production of ROS in chloroplasts and other cellular components (Halliwell & Gutteridge, 2015). Hydrogen peroxide (H_2O_2) and superoxide radicals (O_2•-) are two examples of these ROS. By catalyzing the breakdown of H_2O_2 and other peroxides, peroxidases with their heme groups are essential in reducing this oxidative stress.

Peroxidases can react differently to light stress depending on the type of light, how long they are exposed to it for, and any unique antioxidant defense systems present in them. In order to deal with the elevated levels of ROS, increased light stress may cause the overexpression of peroxidase genes or an increase in the activity of already present peroxidases (Mittler, 2002).

Peroxidase isoenzymes can also modify how they are expressed in response to light exposure, with some isoforms being more sensitive to particular stress conditions than others. According to (Davletova et al., 2005), the control of peroxidase activity and gene expression in response to light stress is a complicated process impacted by the interaction of numerous components and signaling pathways.

In the context of plant and algal biology, understanding how light stress impacts peroxidase responses is essential because it advances knowledge of how cells adapt to oxidative stress and changing environmental conditions.

Non-Heme Peroxidases:

A heme group is absent from the active site of non-heme peroxidases. As an alternative to heme, these peroxidases usually use cofactors or other non-heme iron centers to catalyze peroxidation processes. In contrast to heme peroxidases, non-heme peroxidases frequently contain iron in a different coordination environment, and they have different structural and functional properties. By receiving electrons, they help reduce hydrogen peroxide (H_2O_2) and other peroxides, which is necessary for eliminating reactive oxygen species (ROS) and preventing oxidative cell damage (Arnhold & Flemmig, 2010).

Non-heme different classes and their specific role are mentioned in (Table: 2)

Class	Acronym	Role
▼ **Haloperoxidase superfamily**		In the presence of hydrogen peroxide, haloperoxidases catalyse the oxidative transition of halides (Cl_2, Br_2, or I_2) into XO- (X might be Cl, Br, or I) or organic halogen compounds.
No heme, Vanadium bromoperoxidase	*VBPo*	Certain halo peroxidases require vanadium as vanadate in order for the enzyme to function. A possible HalVPrx is bromoperoxidase (vBPO).
▼**Thiol peroxidase superfamily**		
Fungi-Bacteria glutathione peroxidase	*GPx*	
Plant glutathione peroxidase	*GPx*	In reaction to salt stress, glutathione peroxidase protein or transcript levels rose in plants.
▼**Peroxiredoxin family**		
1-Cysteine peroxiredoxin	*1CysPrx*	only one conserved cysteine in peroxiredoxins. (TSA) proteins, which lessen and detoxify hydrogen peroxide, peroxy nitrite, and organic hydroperoxides, to provide a protective function in cells.
Atypical 2-Cysteine peroxiredoxin (type II)	*PrxII*	homodimeric peroxidase, which is constitutively expressed in the cytosol, peroxisomes, and mitochondria. In these organelles, which are producers of reactive oxygen species, HsPrxV may play a significant antioxidant role in addition to controlling signal transduction.
Typical 2-Cysteine peroxiredoxin	*2CysPrx*	Thiol-specific antioxidant (TSA) proteins reduce hydrogen peroxide, peroxynitrite, and organic hydroperoxides through their peroxidase activity, which offers a protective function in cells.

Table 2 Non-heme peroxidases with different classes and specific roles (Savelli et al., 2019)

Non-Heme Peroxidases' Response to Light Stress

The intensity and duration of light exposure are two variables that can affect how non-heme peroxidases react to light stress. Due to the overproduction of ROS during photosynthesis, light stress, particularly high light intensity, can cause oxidative stress in plant and algal cells. The antioxidative defense mechanism against such oxidative stress involves non-heme peroxidases.

1. Upregulation of Non-Heme Peroxidases: Under conditions of increased light stress, non-heme peroxidases may exhibit an upregulation of gene expression and an increase in enzyme activity. In order to avoid cellular damage, this reaction aids in effectively scavenging and detoxifying ROS, particularly hydrogen peroxide (Ramel et al., 2012).
2. Non-heme peroxidase isoenzymes may react differently to mild stress depending on the isoenzyme. Certain stressors or light intensities may make some isoenzymes more sensitive. According to de Oliveira et al., (2021), these changes in isoenzyme responses contribute to the cell's total antioxidative capabilities.
3. Non-heme peroxidases can interact with other enzymes that are part of the oxidative defense system, including catalase and superoxide dismutase (SOD). According to R. A. Andersen, (2005), this interaction aids in effectively scavenging and neutralizing ROS produced during light stress.

Non-heme peroxidases' reaction to light stress is a component of a larger cellular adaptation to oxidative stress and changing environmental conditions. Understanding how non-heme peroxidases are regulated and how they interact with other antioxidant systems may help us better understand how plants and algae adjust to changing lighting conditions in their natural habitats.

Research Questions & Objectives:

The primary goal of the research is to investigate the potential for achieving high yields of *S. latissima* through the application of using various nutrient solutions. The study aims to identify the optimal nutritional conditions enabling the growth of S. latissima gametophytes under red light stress condition. This research was not intended to be a breeding study, but rather to help guide future breeding practices by investigating how stress conditions affect *S. latissima* growth. Additionally, it aims to investigate the relationship between peroxidase gene expression patterns under red light intensity in terms of stress stimuli in *S. latissima* sporophytes

1. How does the growth pattern of *S. latissima* gametophytes changes when subjected to three different nutrient solutions?

   Objective: Investigate the influence of nutrient solutions on the growth of *S. latissima* gametophytes.

To compare growth pattern of the gametophyte culture I aimed to use three different nutrient solution the growth patterns of *S. latissima* gametophytes will significantly vary when subjected to different nutrient solutions. More specifically, it is anticipated that gametophytes cultures will show varying growth responses based on the particular nutrient composition and concentration of the solutions, and that nutrient-rich solutions will accelerate growth rates relative to nutrient-poor solutions.

2. How does *Saccharina latissima* respond to variations in light stress, both low and high, at molecular level, especially focusing on role of peroxidases?

   Objective: Investigate the changes in gene expression and enzyme activity in sugar kelp under different light stress conditions.

I aim to check the peroxidase activity of the S. latissima in red light stress condition. *S. latissima* responds to variations in red light stress, at both medium and maximum light levels, by modulating the expression of genes related to peroxidases and altering peroxidase enzyme activity. In particular, it is anticipated that red light stress levels conditions could result in variations in peroxidase gene expression and associated alterations in enzyme activity, suggesting a molecular defence mechanism against oxidative stress carried on by red light stress condition.

Materials and Methods

Study 1: Cultivation of S. latissima gametophytes in different nutrient solutions

Description of Experiments

Two studies were conducted to check the effect of nutrient solutions on growth and different light stress effects on *S. latissima* gametophyte.

The **first study started with an experiment** to check the growth of gametophytes in different kinds of nutrient solutions. Three different nutrient solutions were used:

- ES (Enriched Seawater) only consist of nitrogen (N) in the form of sodium nitrate $NaNo_3$ and phosphorus (P) in the form of potassium phosphate KH_2PO_4 nutrients. Culture seawater was sterilized, supplemented with 8×10^{-4} M N and 1×10^{-4} M P
- ½ PES (Provasoli Enriched Seawater) enriched with nutrients, vitaminB1 and Iron-EDTA solution containing trace metal. The precise enrichment details, such as the concentrations of each nutrient added, were provided in the recipe table 03.
- F/2(Guillard's marine Enrichment Medium) enriched with vitamins (B1, B12, H) and trace metal elements. The Recipe table 04 contained the specific enrichment information, and concentration amounts of each nutrient that was added.

The reason to provide poor and rich nutrition is to make a comparison in medium to see growth response. We used three different gametophyte cultures that originate from one spore culture (single cell) each. Two male (M) gametophytes cultures, that looked as small colonies in the top right and bottom well of 6 well MTP and one female (F) gametophyte culture with larger colonies left bottom well (Figure 2), named as

- Sacchagam 19-SpN4 (M)
- Sacchagam 17-SpN2 (M)
- Sacchagam 20-SpN4 (F)

Where 19, 17 and 20 indicates the year in which culture is designated. SpN4 and SpN2 indicates specific genetic line within Collection. From now on SpN4M, SpN2M, and SpN4F respectively.

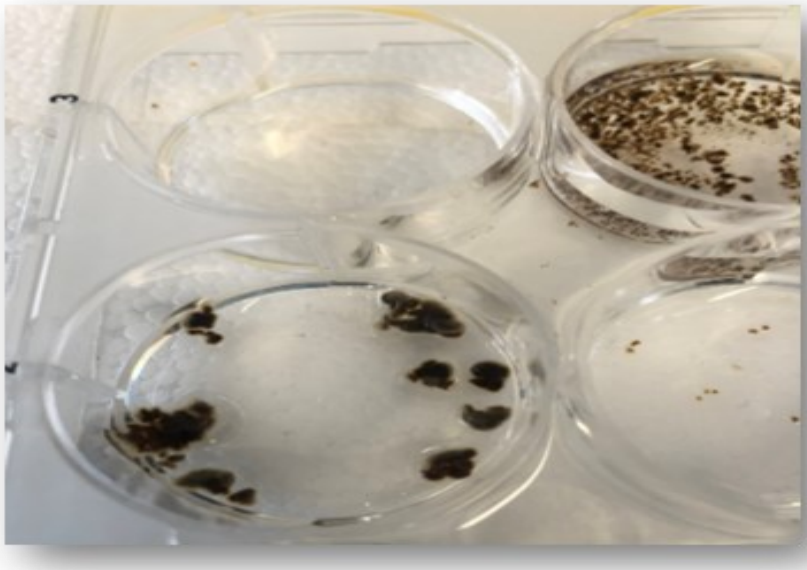

Figure 2 shows *S. latissima* gametophyte cultures, male gametophyte cultures represented by smaller colonies in the top right and top bottom well of 6- well (MTP) and female gametophytes cultures bottom left well with larger colonies (Credit: F. Goecke).

These three different gametophyte cultures were treated with three different nutritional solutions each. Each culture has a replicate with the same solution e.g. Sacchagam 20-SpN4F treated with F/2 solution in two different tubes during the experiment so we can get two measurements with the same nutrient medium and cultures (Figure 3). As the biomass of gametophyte culture was insufficient to create three replicates, only two replicates were made.

Figure 3 *S. latissima* culture treated in cold room with temperature 12°C with different solutions in replicates under red light stress condition.

The **second experiment** was to check the effect of different intensity of light on the gametophytes of *S. latissima* in 1/2Pes nutrient solution. The choice of 1/2 PES over other solutions is due to its well-balanced nutrient profile. 1/2PES is a standard nutrient solution (Boderskov et al.,2022). This standardization allows for reproducible results and easier comparison with existing literatures.

Two different intensities of light were used:

- High intensity light (HL) 280 umol m^{-2} s^{-1}

Low Intensity Light (LL) 20 umol m^{-2} s^{-1}

Media preparation for Nutrition experiment

ES Media (Zhang et al., 2007)

Enriched Seawater (ES) is a weak nutrient medium which only consists of nutrients in the form of Nitrogen (N) and Phosphate (P) in concentrations of 8×10^{-4} M and 1×10^{-4} M, respectively. Nitrogen (N) consist in the form of sodium nitrate $NaNo_3$ and phosphorus (P) in the form of potassium phosphate KH_2PO_4. For 1 liter of culture seawater, 0.068 g of $NaNO_3$ and 0.01361 g of KH_2PO_4 were added to provide the required nutrients. The medium's pH is adjusted to a particular range, typically between 6 and 8. In order to guarantee sterility and avoid contamination during the cultivation phase, the medium is then autoclaved and pre-cooled before using.

½PES Media (R. A. Andersen, 2005)

PES medium is a nickname for provasoli-enriched seawater medium. It contains nitrate and phosphate, as well as TRIS base buffer, trace metals, and vitamins (Table 3).

In preparation of the enrichment stock solution, I started with 900 mL of distilled water and meticulously added each ingredient from Table 03, reserving vitamins for the final stage. After thorough mixing, I diluted the solution to 1 liter with dH_2O, opting for pasteurization (without autoclaving). For the PES medium, I mixed 20 mL of the enrichment stock with 980 mL of filtered seawater, subjecting the mixture to another pasteurization round.

Enrichment Stock Solution

Component	Stock solution	Quantity	Molar Concentration in Final Medium (M)
TRIS base	-----	5.0g	8.26×10^{-4} M
NaNO3	-----	3.5g	8.24×10^{-4} M
Na2 b-glycerophosphate H_2O	-----	0.5g	4.63×10^{-5} M
Iron-EDTA Solution	Recipe table (3.1)	250mL	-------
PII trace metal solution	Recipe Table (3.2)	25mL	-------
Thiamine (vit. B1)	---	0.500mg	2.96×10^{-8} M
Biotin (vit. H)	5.0 mg L-1 dH_2O	1mL	4.09×10^{-10} M
Cyanocobalamin (vit. B12)	10.0 mg L-1 dH_2O	1mL	1.48×10^{-10} M

Table 3 Recipe preparation for enrichment stock for ½ Pes solution

Iron-EDTA Solution

The Iron-EDTA solution was prepared by initiating with a solution of 900 mL of deionized water. I dissolved EDTA in the water, and then I added iron sulfate. Subsequently, I adjusted the final volume to 1 liter. After mixing, I sterilized the solution and stored it in the refrigerator

Component	Quantity	Molar Concentration in Final Medium (M)
$Na2EDTA \cdot 2H_2O$	0.841 g	1.13×10^{-5} M
$Fe(NH4)_2(SO4)_2 \cdot 6H_2O$	0.702 g	8.95×10^{-6} M

Table 3.1 Recipe preparation for Iron solution.

PII Trace Metals

I began by combining 900 mL of dH2O with EDTA to create a solution. Afterward, I individually dissolved the remaining components. Since boron was not necessary, I opted not to enrich natural saltwater with it. I adjusted the final volume to 1 liter, and then I kept the solution cold.

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Component	Quantity	Molar Concentration in Final Medium (M)
Na2EDTA • $2H_2O$	12.74g	1.71×10^{-4} M
FeCl3 • $6H_2O$	0.484g	8.95×10^{-6} M
H3BO3	11.439 g	9.25×10^{-5} M
MnSO4 • $4H_2O$	1.624 g	3.64×10^{-5} M
ZnSO4 • $7H_2O$	0.220 g	3.82×10^{-6} M
CoSO4 • $7H_2O$	0.048 g	8.48×10^{-7} M

Table 3.2 Recipe for PII trace metals

F/2 Media (Ryther, 1962)

It is a regularly and frequently used general enriched seawater medium made for the growth of diatoms and other coastal marine algae. In comparison with the original formulation, known as "f Medium", this has a halved concentration, known as F/2 medium.

I started by adding the following ingredients to 950 mL of filtered natural saltwater. Below were the vitamin and trace element solutions. I used filtered natural saltwater to achieve the final volume of 1 liter. Afterward, I autoclaved the solution.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
NaNO3	75.0 g L^{-1} dH_2O	1 mL	8.82×10^{-4} M
NaH2PO4 H_2O	5.0 g L^{-1} dH_2O	1 mL	3.62×10^{-5} M
Na2SiO3 $9H_2O$	30.0 g L^{-1} dH_2O	1 mL	1.06×10^{-4} M
trace metal solution	Recipe Table (4.1)	1 mL	----
vitamin solution	Recipe Table (4.2)	1 mL	----

Table 4 Media preparation for F/2 solution

f/2 Trace Metal Solution

To have the mixture prepared, I started with 950 mL of dH_2O. I added the ingredients and then topped off the final volume with dH2O to make it 1 liter. After that, I autoclaved the solution. I substituted Na2EDTA and FeCl3 for the ferric sequestrate that was used in the original medium.

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
FeCl3 $6H_2O$	---	3.15g	1.17 x 10^{-5} M
Na2EDTA $2H_2O$	---	4.36g	1.17 x 10^{-5} M
CuSO4 $5H_2O$	9.8 g L^{-1} dH_2O	1 mL	3.93 x 10^{-8} M
Na2MoO4 $2H_2O$	6.3 g L^{-1} dH_2O	1 mL	2.60 x 10^{-8} M
ZnSO4 $7H_2O$	22.0 g L^{-1} dH_2O	1 mL	7.65 x 10^{-8} M
CoCl2 $6H_2O$	10.0 g L^{-1} dH_2O	1 mL	4.20 x 10^{-8} M
MnCl2 $4H_2O$	180.0 g L^{-1} dH_2O	1 mL	9.10 x 10^{-7} M

Table 4.1 Recipe for F/2 trace metal solution for F/2 solution

f/2 Vitamin Solution

First, I obtained the primary stock solutions. Then, to create the ultimate vitamin solution, I mixed thiamine in 950 mL of deionized water (dH_2O). Subsequently, I added the primary stocks in the amounts listed in the quantity column below. Finally, I added dH2O to bring the final volume to 1 liter. Afterward, I sterilized the solution by autoclaving. The solution was then placed in the freezer.

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Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine HCl (vit. B1)	----	200 mg	2.96 x 10^{-7} M
biotin (vit. H)	0.1 g L^{-1} dH^2O	10 mL	2.05 x 10^{-9} M
cyanocobalamin (vit. B12)	1.0 g L^{-1} dH^2O	1 mL	3.69 x 10^{-10} M

Table 4.2 Recipe Preparation for f/2 vitamin solution

Indoor culture and experimental design:

The vegetative amplification of gametophyte clones was carried out in a temperature-controlled cool chamber equipped with a red-light stress condition. Gametophyte clones were cultivated in flasks (approximately 0.75l of the flask was used) and continually bubbled with filtered air. A glass pipette with a 3.0 mm aperture was used to give aeration in the flask's center, near the top. The aeration intensity was sufficient to suspend the gametophyte fragments without creating significant turbulence. The bottles had been sealed to avoid contamination(Zhang et al., 2007).

Cultivation of Gametophyte.

The following preparatory steps were done in order to measure the growth rate of gametophyte culture with various media:

For sample collection gametophyte culture were collected from a larger flask growing in cold room with seawater medium under red light. The culture was filtered out by a filter sieve.
The wet weight of the filtered gametophytes was measured. To ensure accuracy, a hand pump (vacuum) was used to remove excess water from the samples. It was crucial not to extract too much water to prevent desiccation, as excessive drying could negatively impact the gametophytes during the weighing process.

The entire gametophyte culture was divided into six parts (Figure 4). This division ensured that each solution had a replicate, with the setup being: solution 1*((3) gametophyte cultures * (2) replicate). Due to the limited biomass of the gametophyte culture, it was feasible to prepare only two replicates for each condition, as three replicates could not be achieved.

Two flasks were used to culture each gametophyte culture and media, acting as true replicates for the study. It was ensured that any effects on growth rates that were detected could be attributable to the experimental settings rather than random variability by using genuine replicates.

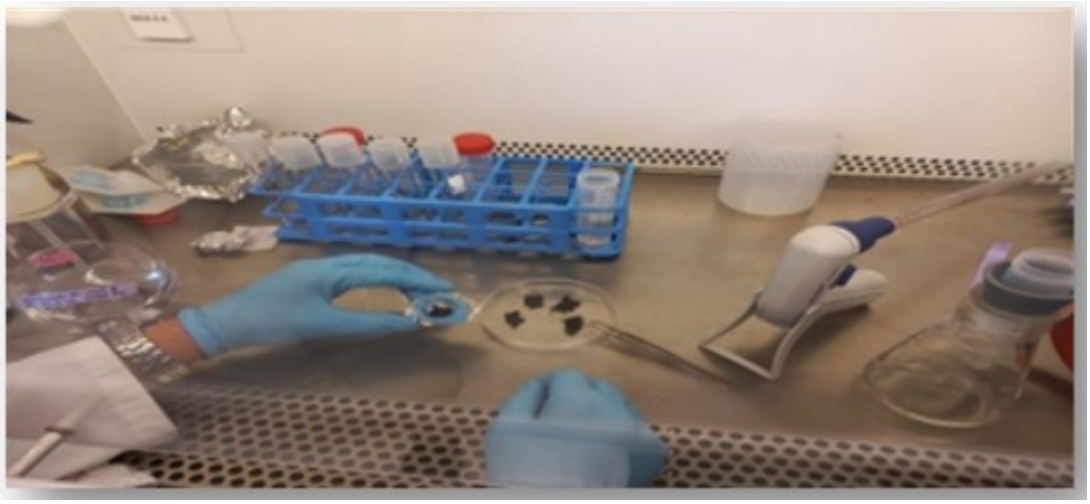


Figure 4 Division of gametophyte culture under laminar flow.

Measurement of Wet Weight:

Each part of the gametophyte culture was weighed using an extremely sensitive balance, as the culture quantity was small, and minor changes in milligrams could affect the final value.

After weighing the wet weight, each culture was placed in an individual tube containing 30 ml of the solution (ES, 1/2 PES, F/2). Accurate labelling was done to avoid any confusion during the results collection.

The labelled tubes were grown in cold room with 12°C under red light stress-controlled conditions (Figure 3). The wet weight of gametophyte clones was measured at 10-day and 20-day intervals. Before measurement, the medium was removed using a hand pump and a nylon filter with a 60-µm aperture, following the same procedure each time. The entire experiment was conducted under laminar airflow (Figure 5) to ensure smooth operations and to protect the media from any contamination.

The culture was refreshed with a new medium after 10, 30, and 50 days. The biomass was measured each time to maintain consistent nutrient conditions and to avoid any nutritional deficiencies that could alter the results. The culture was kept alive for more than 70 days. The mass increases of gametophyte culture in wet weight was used to perform statistical analysis, e.g. to calculate the relative growth rate (RGR).

Statistical Analysis.

Statistical analyses were performed using R Studio (R Team, 2023) and Microsoft Excel. Gametophyte growth was checked by various analysis of growth relations e.g.; Relative Growth Rate, Cumulative Growth Rate. The fresh weight of gametophyte culture was measured at 10, 30, and 50 days since the beginning of the experiment. Based on those readings different statistical analyses were performed e.g. ANOVA

Relative growth rate (Hunt, 2003):

The difference between an individual's size at the beginning of the period and its size after a brief interval is known as the relative growth rate, or RGR.

RGR is a numerical value that evaluates the proportionate increase in an organism's size, mass, or other metric relative to its starting state. It is expressed as a rate per unit of time.

Relative Growth Rate Formula:

Relative growth rate (RGR) is determined in traditional growth analysis as

$$RGR = \frac{\ln(w2) - \ln(w1)}{T2 - T1}$$

where W1 and W2 are plant fresh weights at times t1 and t2, respectively.

The RGR graph depicts growth in a dynamic manner by displaying growth rates at certain time intervals (e.g., days 10, 30, 50). RGR assists in identifying temporal trends that indicate times of rapid or decelerating growth.

Cumulative Growth Rate:

The term "cumulative growth rate" (CGR) refers to a measurement that accounts for the cumulative growth at each time point and is used to quantify the overall growth attained over a certain period of time. It indicates the total proportionate increase in size or mass and is frequently stated as a percentage or ratio.

Cumulative Growth Rate formula

Cumulative growth formula

$$CGR = \frac{Wf - Wi}{Wi}\ 100$$

Where Wf is the final weight on day 10 and Wi is the initial weight on day 0ne. The numerator (Wf-Wi) is the absolute increase throughout the stated period, while the denominator (Wi) is the original size, which serves as a baseline for comparison.

The Cumulative Growth graph provides a comprehensive view by displaying the overall growth accumulation over the course of the experiment. This cumulative lens enables us to examine not just the peaks and troughs observed in instantaneous growth rates, but also the overall influence of these fluctuations on our organisms' total size or mass.

Analysis of Variance (ANOVA):

ANOVA is used to determine whether there are any statistically significant differences in the means of two or more independent groups. It aids in determining whether there are substantial changes in culture increase across different solutions or time periods.

Two-way ANOVA analysis was conducted to assess the impact of both different solutions and different cultures on the growth responses of *S. latissima*.

Experimental Design and Culturing of Light Intensity Experiment:

To begin with this experiment gametophyte cultures were collected from flasks and put in a petri dish plate, after removing water from the hand pump, the whole culture was then divided into 8 parts (Figure 4). Half culture was put in high-intensity light HL (280 umol m^{-2} s^{-1}) and half in low-intensity light LL ((normal gametophyte light culture) 20 umol m^{-2} s^{-1}). The algae had at least time to rest as it had a light regime of 8 hours dark and 16 hours day, and it ran for 8 days. It was made at the red room (under 12 ˚c), but we used extra an air ventilator next to it to avoid an increase of temperature. For each light intensity two replicates were used. We used a similar initial weight for each culture and replicates (between 180mg to 260mg). Each culture was placed on a 6-well MTPs (multi-well tissue culture plate) plate randomly with a solution medium ½ PES. (Figure 5)

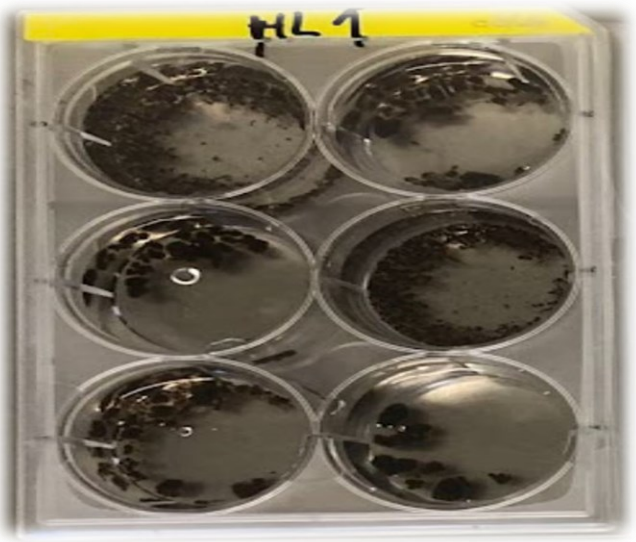


Figure 5 showing different gametophyte cultures put randomly in a 6-well MTPs plate.

The same procedure is repeated with the other four gametophyte cultures. In this way we have four 6-well MTPs plates for high light (HL) and four plates for low light (LL) (Figure 6)

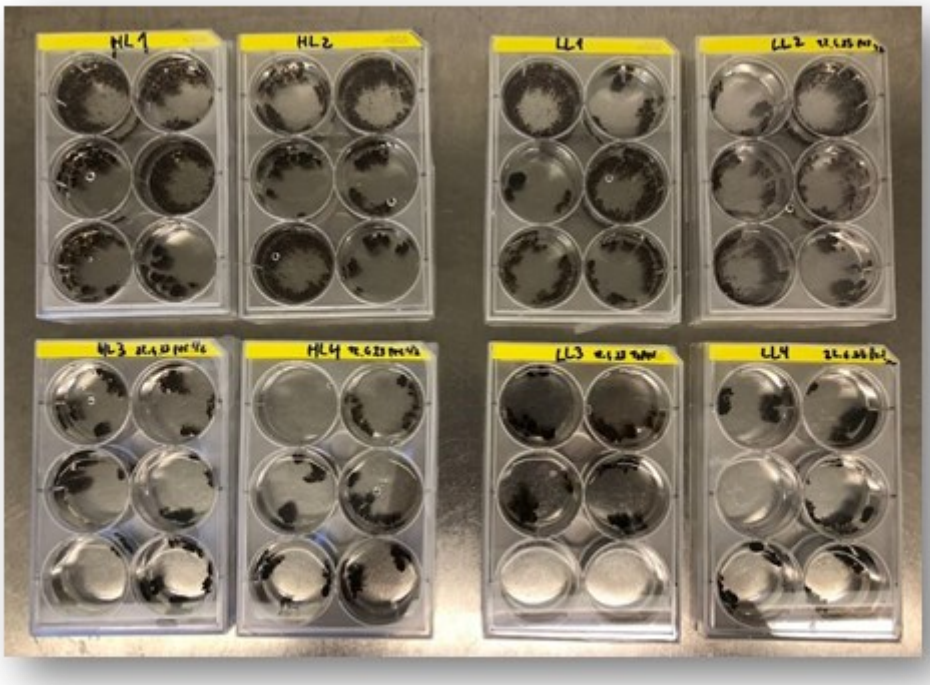


Figure 6 Shows 8 plates 6-well MTPs 4 for high light experiment names HL and 4 plates for low light named LL.

After 8 days of exposure of samples to different light, each of the cultures was put in aluminum foil like a package form individually and put in liquid nitrogen so we could freeze it for further RNA extraction.

As we are bringing plates from the cold room to the laboratory there is a risk that samples get heated from the environment. The plates were put in an isopor box carefully with a cooling device which helps to maintain the temperature as in the cold room. To avoid darkness a red-light source was provided in an isopor box and packing was done so quickly, to not to have exposure to white light for long time (Figure 07). This experiment was not fully completed due to shortage of time. It involved RNA extraction and preservation of samples for future studies.

Figure 7 (A) the samples treated with different light in cold room (B) Packaging of the sample for freezing (C) Ideal condition with light and cooling device in isopor box.

RNA Extraction

The RNA-extraction methodology was derived from the study conducted by (Heinrich et al., 2012), with minor adjustments. Prior to initiating the experiment, four volumes of 100% ethanol (44 ml) were used to dilute the buffer RPE. Furthermore, the DNase I stock solution was made ready for a later on-column DNase extraction. 550 µl of RNAse-free water was used to dissolve 1500 Kunitz units of lyophilized DNase I. After being taken out of the vial, the DNase I stock solution was aliquoted into 1.5 ml tubes and kept in a freezer at -20 °C.

Using a TissueLyser (4x 30 seconds at 25 Hz), 100–150 mg of frozen material was transferred to 2 ml sample tubes containing QIAGEN tungsten carbide beads. Liquid nitrogen was used to keep samples frozen until the extraction buffer was added. Twenty microliters of 2 M DTT and one milliliter of extraction buffer (100 mM Tris pH 8, 1 M NaCl, 50 mM EDTA pH 8, 2% CTAB) were added to the tubes. After carefully vortexing the samples, they were incubated for ten minutes at 45 °C. After adding

one milliliter of chloroform: isoamyl alcohol (24:1), the tubes were centrifuged for 20 minutes (12000 x g, 20 °C) and shaken for ten minutes at maximum speed.

Aqueous phase (750 µl) was transferred to fresh 2 ml tubes. After adding 1/3 volumes (about 225 µl) of 100% EtOH, the tubes were gently inverted upside down to combine the samples. After adding 1 volume, approximately 975 µl, of chloroform:isoamyl alcohol (24:1), the samples were centrifuged for 20 minutes (12000 x g, 20 °C). One volume (600 µl) of chloroform was added to fresh tubes containing 600 µl of the aqueous phase. The samples were centrifuged at 12000 x g for 10 minutes at 20 °C. The aqueous phase (450 µl) was poured into fresh tubes.

After that, the extraction was carried out using the QIAGEN RNeasy kit in accordance with the manufacturer's protocol (pp. 62–66), beginning at step 3 (QIAGEN, 2012). After adding 450 µl of buffer RLT to each sample tube, the tubes were vortexed. After transferring the lysate from each sample tube to a QIAshredder spin column that was put in a 2 ml collection tube, the tubes were centrifuged for 2 minutes at 12000 x g and 20 °C. This operation was done in two steps because the lysate was more than the QIAshredder spin columns' 650 µl limit.

Each sample's flow-through supernatant was poured into a fresh 2 ml collecting tube. The majority of the debris was eliminated during the chloroform extraction, so there was no pellet visible at the bottom of the tubes, but the bottom was still undisturbed. To the lysate, 0.5 volumes of 100% ethanol (around 400–450 µl) were added and pipetted together. At this stage, the tubes were not centrifuged or vortexed. After that, the lysate was moved to RNeasy spin columns that were positioned in 2 ml collecting tubes.

After centrifuging the samples for 15 seconds at 8000 x g (20 °C), the flow-through was thrown away. Because sample lysate quantities surpassed 650 µl, this procedure was repeated twice for each sample (although collected in the same spin column). At this stage, the same technique for on-column DNase digestion was followed (protocol page. 82–84). Firstly, the RNeasy spin columns were filled with 350 µl of buffer RW1. After centrifuging the samples for 15 seconds at 8000 x g (20 °C), the flow-through was thrown away. For each sample, a DNase I incubation mix was prepared by combining 10 µl of DNase I stock solution with 70 µl of buffer RDD. Following the direct pipetting of 80 µl of incubation mix onto the spin column membrane for each sample, the samples were allowed to sit on the benchtop at about 22 °C for 15 minutes.

Following the incubation period, the spin column membrane was cleaned by centrifuging the samples for 15 seconds at 8000 x g (20 °C) and adding 350 µl of buffer RW1. The through-flow was abandoned. Since step 7 is an additional RW1 wash step, it was skipped upon returning to the main protocol. Next, the RNeasy spin columns were filled with 500 µl of buffer RPE. Although the centrifugation speed and duration in this phase were changed throughout the experiment, the protocol called for starting at 8000 x g for 15 s (20 °C).

However, the speed and length were increased to 8500–9000 x g for 18–20 s (20 °C) in order to address some impurities found in the final product. The phase was then repeated for a few cycles. Subsequently, 500 µl of RPE buffer was added to the spin columns, and the samples were centrifuged at 8000 x g for two minutes at 20°C to further wash and dry the spin columns. The old collection tubes with flow-through were thrown away and the spin columns were transferred into brand-new, 2 ml collection tubes. After that, the samples were centrifuged at 12000 x g for 10 min at 20 °C.After the spin columns were placed in brand-new 1.5 ml collecting tubes that came with the kit, 30 µl of RNAse-free water was put right on top of them. Prior to centrifugation, the samples were placed on the benchtop for one minute. They were centrifuged at 8000 x g for one minute at 20 °C, yielding a final eluate that was flow-through. A 3.5 µl aliquot of each sample was reserved for testing with TapeStation and NanoDrop. While the TapeStation analyzes the quantity and integrity of the RNA (Agilent Technologies Inc, 2015), NanoDrop aids in evaluating the quantity and purity of the RNA samples (Thermo Fisher Scientific Inc., 2008). The samples will be kept in a freezer at -80 °C until they are sent to NoVo Gene for RNA sequencing.

Study 2: Transcriptome analysis of light-stressed S. latissima sporophyte with respect to peroxidases

Sampling

Individuals were grown on ropes at a Seaweed Solutions farm off the coast of Frøya and sampled by Silje Forbord and Margot Nyeggen in May 2021. Individuals of *S. latissima* with a length of about 60 cm were chosen. After harvesting, the thalli were shipped to SINTEF's Ocean Trondheim, where they underwent a period of acclimatisation, as detailed in the section below.

Experimental design:

In this crossed-design experiment, tissues were exposed to light at 100 or 250 µmol $m^{-2}s^{-1}$ for one, three, or nine days with light exposure 24 hours per day. Each sporophyte had two tissue sections (15 cm each) cut from the central region above the meristem and randomly placed in beakers. They were acclimated to 40 µmol light in flow-through deep water at 10 °C. Four complete sporophytes were also acclimated in the same manner. Before the trial started, there was a seven-day period of acclimatization.

Table 05 shows that six treatment groups (100 day 1, 250 day 1, 100 day 3, 250 day 3, 100 day 9, and 250 day 9) and control groups (day 0 uncut, day 0, day 1, day 3, day 9). The control groups were exposed to the same light as during the acclimation phase.

Light Intensity	Day 0	Day 1	Day 3	Day 9
MIN (40 µmol light)	C0 and UC (uncut)	C1	C3	C9
MED (100 µmol light)		MED1	MED3	MED9
MAX (250 µmol light **)**		MAX1	MAX3	MAX9

Table 5: Setup for the transcriptome investigation on individuals of *S. latissima* under light stress. The following are abbreviations for control days: C0, C1, C3, and C9. The following are the sample numbers: MED1 = 100 µmol $m^{-2}s^{-1}$ light for 1 day, MED3 = 100 µmol $m^{-2}s^{-1}$ light for 3 days, MED9 = 100 µmol $m^{-2}s^{-1}$ light for 9 days, MAX1 = 250 µmol $m^{-2}s^{-1}$ light for 1 day, MAX3 = 250 µmol $m^{-2}s^{-1}$ light for 3 days, and MAX9 = 250 µmol $m^{-2}s^{-1}$ light for 9 days. All controls were exposed to 4 µmol $m^{-2}s^{-1}$ light

The denovo assembly and the transcriptomic data from Simon Haughom thesis (2022) was further used to study the effects of peroxidases genes in *S. latissima.*

Mining of Peroxidases from Databases

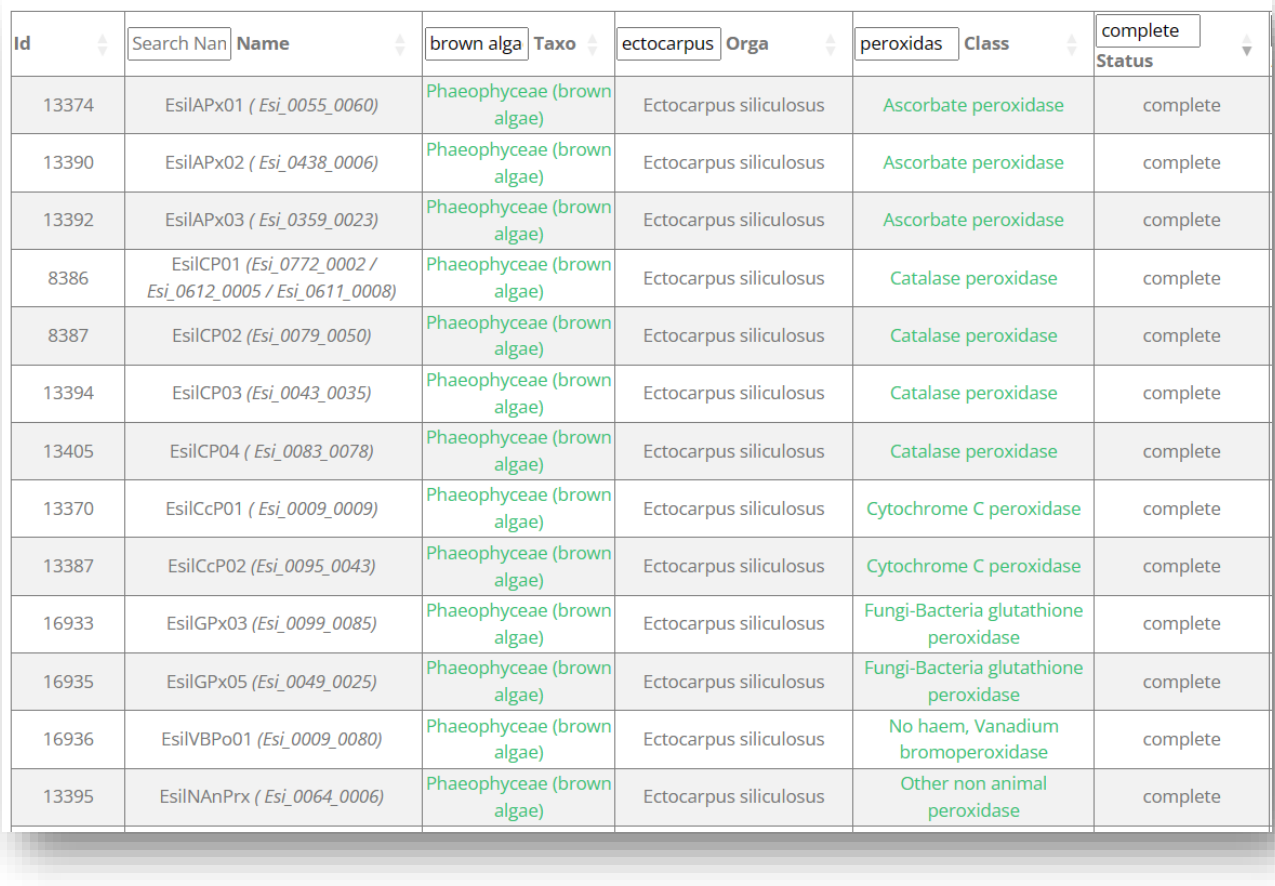
Due to the unavailability of the complete genome sequence of *S. latissima*, there is no annotation information available for this species. We do not know much about the peroxidase genes in *S. latissima* to understand more about their role. So, we must rely on closely related brown macroalga species e.g. *Ectocarpus siliculosus* and *Saccharina japonica*, to identify homolog sequences of peroxidases in *S. latissima.*

In order to mine the peroxidases, from the Phaeophyceae, we searched this key term in the database named RedoXiBase (Savelli et al., 2019). This is a vast database that has 1000 organism's data set present in RedoXiBase and they are distributed in all kingdoms based on NCBI taxonomy. To investigate this database certain parameters were used.

Parameters:

After Phaeophyceae was selected under the taxonomic group Stramenopiles, 40 organisms were sequenced according to NCBI TaxId. Out of the 40 different organisms, only one organism, *Ectocarpus siliculosus*, had 62 peroxidases that were completed or uncompleted sequenced. Further parameters were set in the following way (Figure 8)

- Taxonomic group (Phaeophyceae (brown algae))
- Organisms name (*Ectocarpus siliculosus*)
- Classes (Peroxidases)
- Sequence status (Completed



Id	Search Nan Name	brown alga Taxo	ectocarpus Orga	peroxidas Class	complete Status
13374	EsilAPx01 *(Esi_0055_0060)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Ascorbate peroxidase	complete
13390	EsilAPx02 *(Esi_0438_0006)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Ascorbate peroxidase	complete
13392	EsilAPx03 *(Esi_0359_0023)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Ascorbate peroxidase	complete
8386	EsilCP01 *(Esi_0772_0002 / Esi_0612_0005 / Esi_0611_0008)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Catalase peroxidase	complete
8387	EsilCP02 *(Esi_0079_0050)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Catalase peroxidase	complete
13394	EsilCP03 *(Esi_0043_0035)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Catalase peroxidase	complete
13405	EsilCP04 *(Esi_0083_0078)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Catalase peroxidase	complete
13370	EsilCcP01 *(Esi_0009_0009)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Cytochrome C peroxidase	complete
13387	EsilCcP02 *(Esi_0095_0043)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Cytochrome C peroxidase	complete
16933	EsilGPx03 *(Esi_0099_0085)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Fungi-Bacteria glutathione peroxidase	complete
16935	EsilGPx05 *(Esi_0049_0025)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Fungi-Bacteria glutathione peroxidase	complete
16936	EsilVBPo01 *(Esi_0009_0080)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	No haem, Vanadium bromoperoxidase	complete
13395	EsilNAnPrx *(Esi_0064_0006)*	Phaeophyceae (brown algae)	Ectocarpus siliculosus	Other non animal peroxidase	complete

Figure 8: show the complete sequenced searched peroxidases from *Ectocarpus siliculosus*.

I collected all the peroxidases that have complete sequences.

Once collected the sequences, I performed the BlastN against our transcriptome database (Simon 2022) to find homologous sequences. The following are the parameters to Run the BlastN

Step 1: Prepare BLAST query and Database

Before running BLAST, it was made sure to have:

- FASTA file contain genes sequences.
- A transcriptome *S. latissima* database (e.g., NCBI's nr database) or a custom database prepared with the target sequences.

Searched *E. siliculosus* peroxidase sequences were arranged in FASTA format to run BlastN. A software tool **PuTTy** was used to connect to HPC cluster (Orion) to run BlastN using Blast+ 2.11.0 version.

Step 2: Create a SLURM Script

To create a SLURM, a script (a batch job script) was needed that specifies the BLAST job details, resources, and how it should be executed. Here's a sample script: (Figure: 11)
Here's what each line does:

- **#SBATCH** lines are SLURM directives that specify job settings **(-J)** BLASTDATABASE **(-n)** number fo CPUs **(-N)** node
- **module load blast+/2.11.0** loads the BLAST module, latest version.
- **blastN** is the BLAST program that I want to run, and specify the query (**-query**), give path to FASTA file.txt database (**-db**) db type is nucleotide nucl, output file (**-out**), out format (-**num_alignments**) number of alignments 6, and the number of threads (**-num_threads**) 5.

Step 3: Submit the Job

Once the SLURM script was ready, saved it (e.g., as **blast_job.sh**) and submitted it using the **sbatch** command. This command submits the job to the cluster, and SLURM will allocate the specified resources and run the BLAST job.

Step 4: Monitor the Job

It can monitor the job's status using **squeue** or **sacct** commands
Once the job is completed, the results will be in the **blast_output.txt** file specified.

```
#!/bin/bash

## run with sbatch job_script

# Job name:
#SBATCH -J BLASTDATABASE
# number CPUs to be used
#SBATCH -n 20
#SBATCH -N 1
#SBATCH -o transcriptome_blastdatabse.out

module load BLAST+/2.11.0-gompi-2020b

#myINPUT=/net/fs-1/IPVProjects01/ipv0/POPULUS_RNASEQ/RNAseq_Data/seq_assembly/Trinity_Populus_Two.Trinity.fasta
#myHOME=/net/fs-1/IPVProjects01/ipv0/POPULUS_RNASEQ/RNAseq_Data/seq_assembly

#Script to make own blastdatabase

makeblastdb -in /mnt/users/rabyashf/transcriptome/safekelp_trinity_assembly.Trinity.fasta -dbtype nucl -out /mnt
index -parse_seqids
```

Figure 9 showed command lines for BlastN.

Gene expression of the peroxidases under different light conditions

To examine the expression of peroxidase genes in *S. latissima* under different light conditions, we found homologous genes using known peroxidase sequences from *E. siliculosus*. The *E.siliculosus* peroxidase gene sequences were used as queries in a BLAST search against the *S. latissima* transcriptome to find potential homologs. RNA-Seq data for *S. latissima* were collected from samples exposed to various light conditions: C0, C1, C3, C9, Med1, Med2, Med3, Max1, Max2, and Max3. These conditions indicate a progression of light stress levels. The raw sequencing reads were quality-checked with FastQC, before being trimmed to remove low-quality bases and adaptor sequences with Trimmomatic. The processed reads were then aligned using the HISAT2 aligner, the quality-filtered RNA-Seq reads were mapped to the transcriptome of *S. latissima*. SAM files were produced during the alignment procedure. These files were sorted using SAMtools and converted to BAM format for further analysis. To quantify gene expression levels, we used the Subread package's featureCounts utility. This utility calculated raw read counts for each gene by counting aligned reads in BAM files. To identify differentially expressed genes among the peroxidase gene homologs under different light conditions, we employed the DESeq2 package in R, which performs differential expression analysis using the negative binomial distribution. Raw read counts from featureCounts were imported into DESeq2, creating a DESeq2 dataset object (DESeqDataSet) that incorporated sample information, including light conditions (C, MED and MAX). DESeq2 normalized the read counts to account for library size differences and estimated gene-wise dispersions to model expression variability. Pairwise comparisons between different light conditions were conducted to identify differentially expressed peroxidase genes. We used R's ClustVis utility to visualise peroxidase gene expression patterns under various light conditions. Principal Component Analysis (PCA) plots were made to investigate the variation in gene expression, and heatmaps were created to show the expression levels of the peroxidase genes under different situations.

Results

Study 1: Cultivation of S. latissima gametophyte culture with different nutrient solutions

Effect of nutrient solution on gametophyte growth:

Relative growth rate:

Figure 10 shows the relative growth performance of 3 different gametophyte cultures during 50 days of observations.

Firstly, when comparing three solutions of culture 17SPN2M, it was observed that this culture showed a similar growth rate in two solutions, 1/2PES and F/2. In solution ES, there was an initial negative growth in the first 10 days, followed by a relatively modest upward trend from day 30 to 50. However, this increase was not as significant as observed in the other two solutions.

In the 1/2PES solution, the growth rate was nearly zero initially, then experienced a drastic increase over time. In the ES solution, there was zero growth initially, with a slight increase observed until the end of the experiment. In the F/2 solution, the growth rate remained almost constant throughout the 50-day duration. For the 20SpN4F culture, the growth rate in the first 10 days was good, but after 10 days, the growth rate decreased. After 30 days, the growth rate started increasing again. In the ES solution, the growth rate gradually decreased but remained positive. In the F/2 solution, the growth rate increased throughout the 50 days.

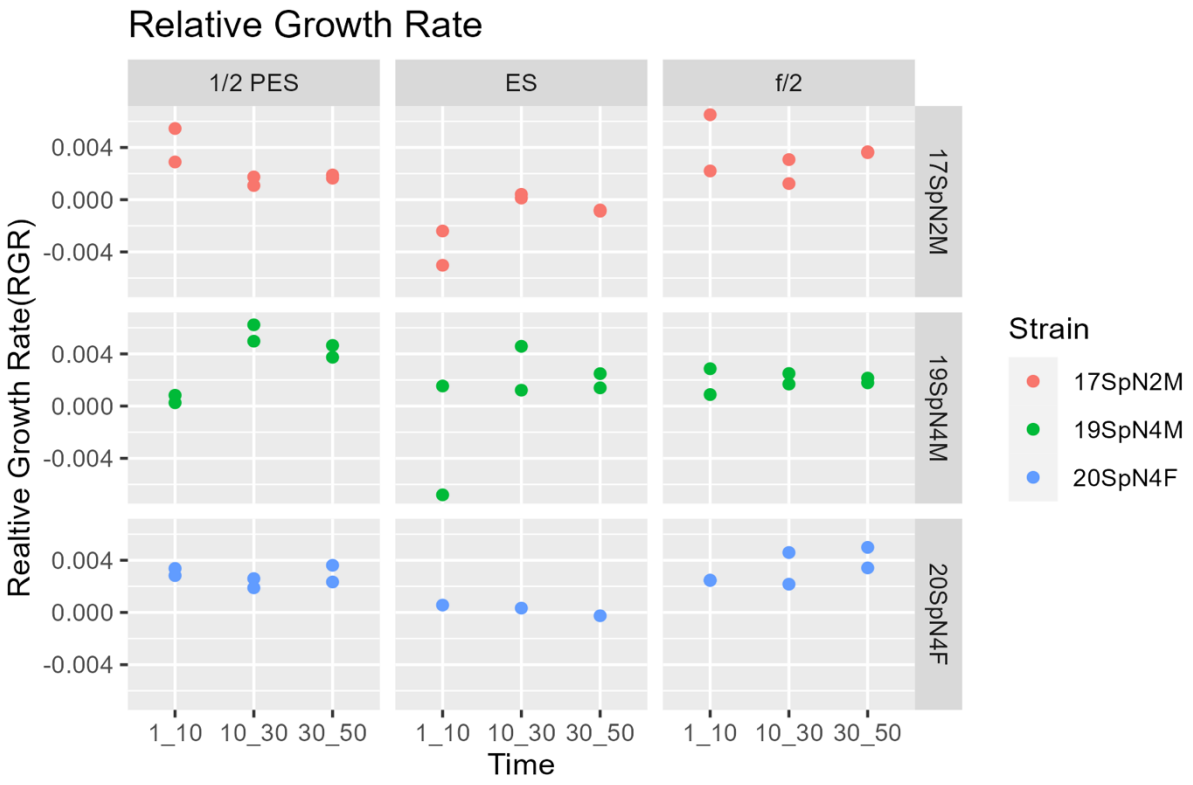


Figure 10: Relative growth rate of gametophyte cultures grow in different nutrient solutions.
1-10 show growth from day 1 to day 10. 10-30 shows growth from day 10 to day 30 and 30-50 shows growth from day 30 to day 50. Each graph shows 2 reading with respect to one time periods are the replicates of specific cultures in the particular solution.

Cumulative Growth Rate:

When examining the graph 2 with respect to the solution, the 1/2PES solution showed linear growth after 10 days, while 19SpN4M exhibited very significant growth after 10 days. The 20SpN4F culture showed continuous growth throughout the 50 days. In the F/2 solution, all three cultures grew continuously; however, 19SpN4M did not grow as much compared to 17SPN2M and 20SpN4F. The 20SpN4F culture exhibited abrupt growth after 30 days. In the ES solution, the cultures grew differently. The 17SPN2M culture showed negative growth throughout the entire time period, while 19SpN4M had negative growth in the first 10 days but showed positive growth after that. The 20SpN4F culture showed very little growth, almost equal to zero.

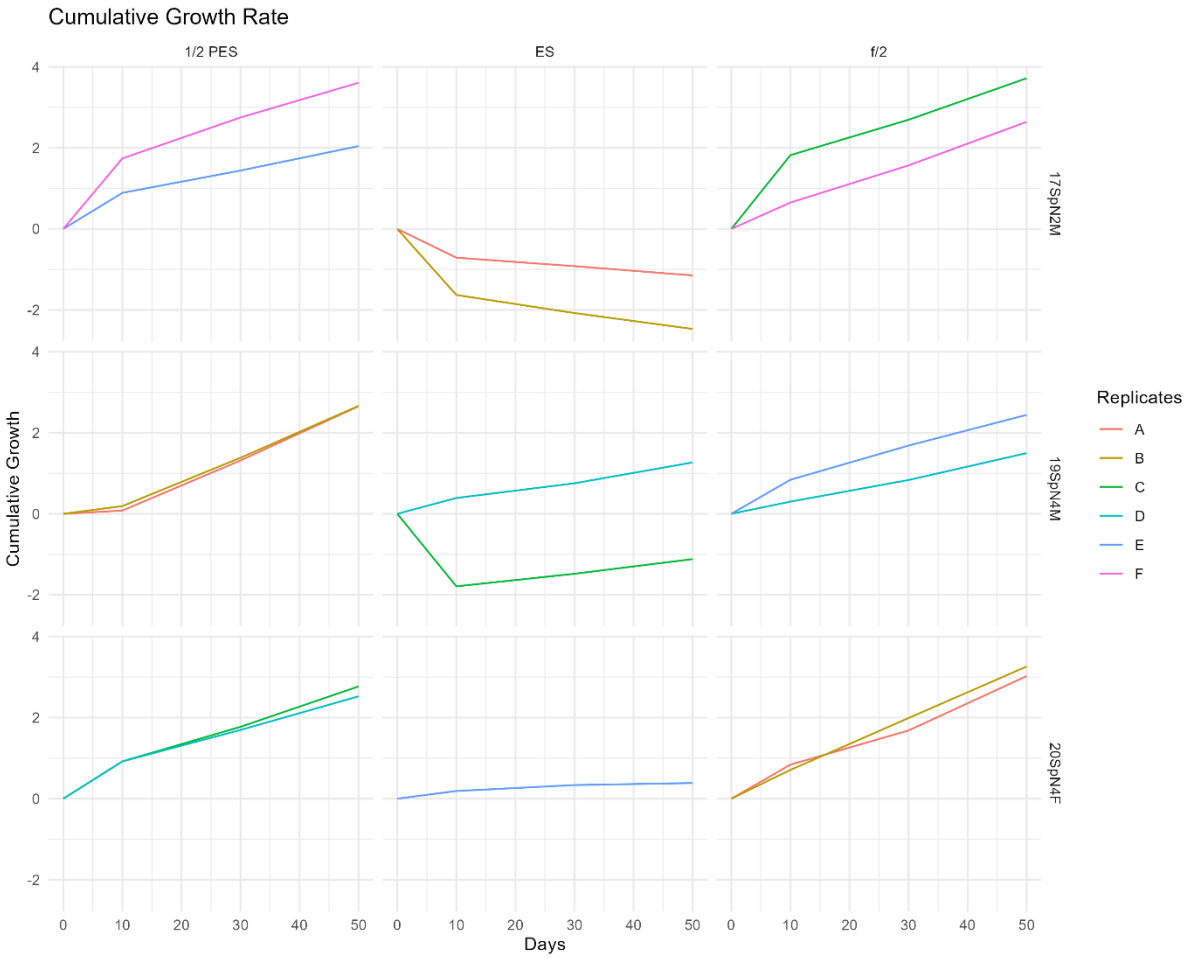


Figure 11: Cumulative Growth rate of Gametophyte Cultures related to Different solutions.

Two-way repeated measures ANOVA with respect to solution and time effect on growth:

In the table 6: three effects are being tested: the main effect of Solutions, the main effect of time, and the interaction effect between Solutions and time

	DFn	DFd	F value	P value	Generalized Eta square (ges)
Solutions	2	4	7.938	0.040	0.551
Time	2	4	5.737	0.067	0.407
Solutions:Time	4	8	0.656	0.639	0.096

Table 6: results from two way ANOVA Degrees of Freedom (DFn) Degrees of Freedom (DFd) F-value, p-valu, Generalized Eta Squared (ges).) The numbers stated are p-values with significance levels: p=0.05*, p=0.01**, p=0.001***

1. Main Effect of Solution

The major effect of solutions on growth was found to be statistically significant (p = 0.040). This suggests that the type of solution used had a substantial effect on growth. The generalized eta squared (ges) indicated an effect size of 0.551, which appeared to be large. This implies that variances in solutions accounted for a significant amount of the variety in growth.

2. Main Effect of Time

There was no apparent main effect of time on growth (p = 0.067). Although the p-value was close to the threshold for significance, it did not meet the standard cutoff of 0.05. The effect size (ges = 0.407) was medium, indicating that time had a moderate impact on growth; however, this effect was not statistically significant in this case.

3. Interaction Effect of Solution and Time

There was no statistically significant interaction effect of solutions and time on growth (p = 0.639). This means that the effect of one variable on growth was independent of the other variable's level. The effect size (ges = 0.096) was small, implying that the interaction of solutions and time accounted for only a small amount of the variance in growth.

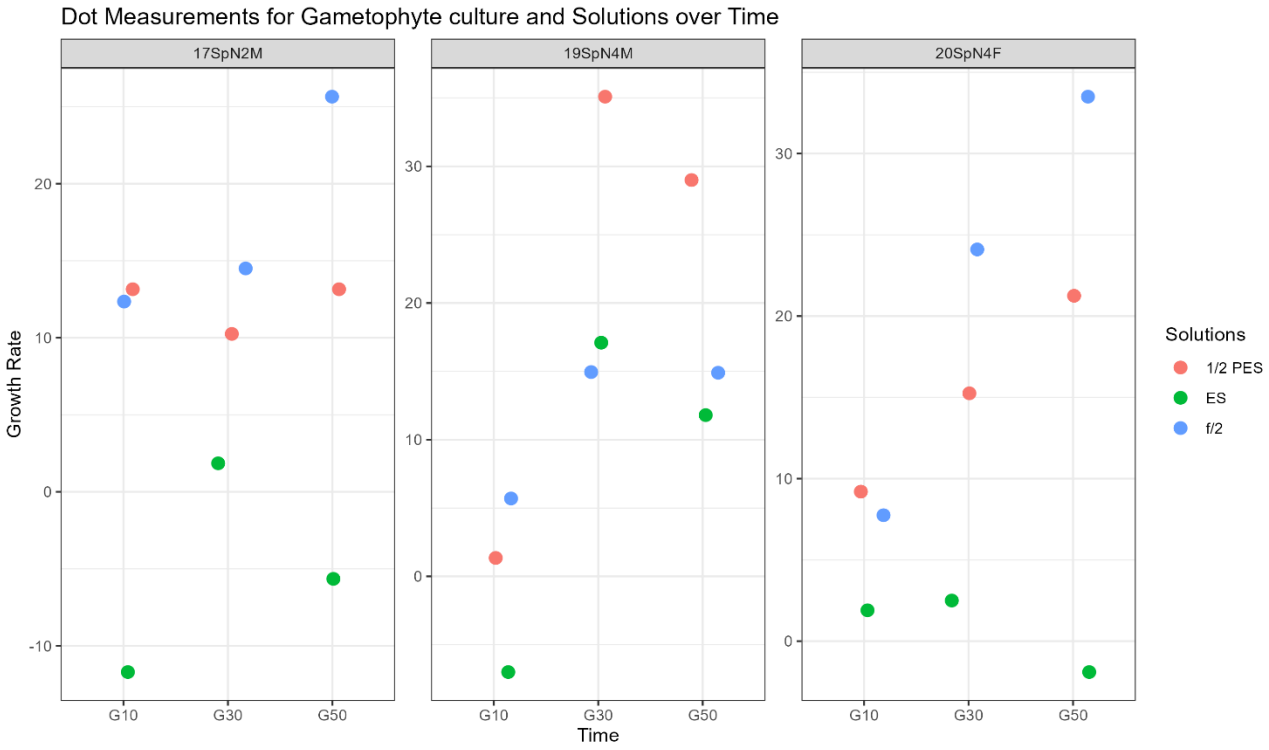


Figure 12: Two-way repeated measurement ANOVA visualization with respect to solution and time effect on gametophyte growth.

Study 2: Transcriptome analysis of light-stressed S. latissima sporophyte with respect to peroxidases

Distribution of Peroxidases among Groups

The distribution of the peroxidase gene families of *E. siliculosus* collected from the Redox database is shown in the pie chart. Different types of 27 heme and non-heme peroxidase IDs from *E. siliculosus* are collected, from 11 groups e.g. 1 cysteine peroxiredoxin, ascorbate peroxidase, catalase peroxidase, Peroxidasin etc.

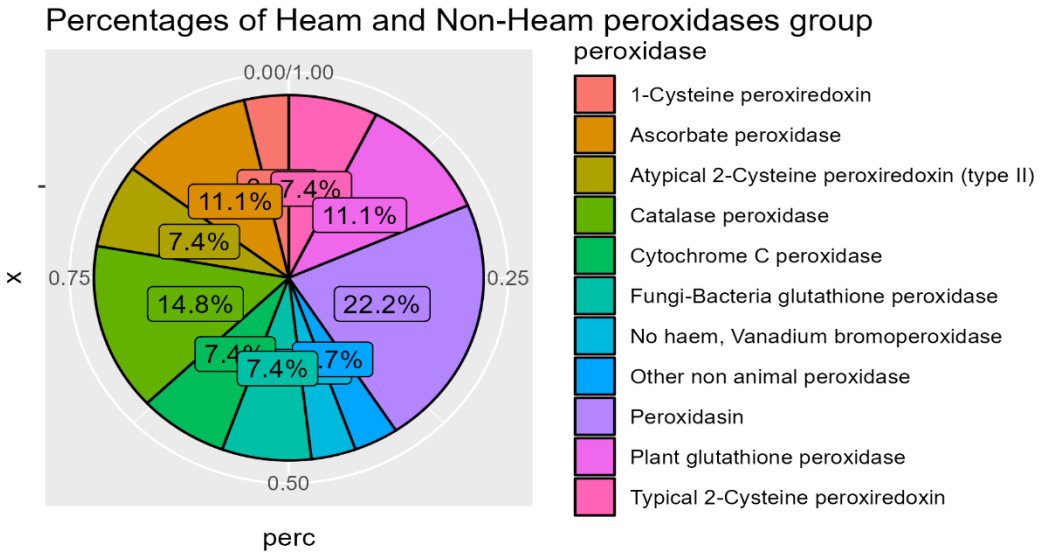


Figure 13: show the percentage of peroxidase genes have more hits with transcriptomic data

Interpretation of the Pie Chart:

- The pie chart visually represents the distribution of peroxidases from different groups.
- Each slice of the pie represents a different peroxidase group.
- The size of each slice corresponds to the percentage of peroxidases within that group relative to the total.

For Example. Peroxidasin has the highest percentage 22% and the 1-Cysteine peroxiredoxin, No-Heam vanadium Bromo peroxidase, and other non-animal peroxidases have the lowest 3.7%. This will help in finding out which peroxidases have more hits in our dataset.

Identification of peroxidases homologous genes in S. latissima

Ectocarpus Peroxidases in *S. latissima* Transcriptomic Data

After running a BlastN analysis, a total of 186 hits were found for *E. siliculosus* peroxidases that matched *S. latissima* trinity IDs. The percentage of matching ranged from 65% to 92%. This percentage represents the degree of sequence similarity or identity between the peroxidase genes of E. siliculosus and the corresponding sequences in S. latissima. In other words, the sequences from S. latissima matched with those of E. siliculosus peroxidases by 65% to 92% in terms of nucleotide sequence alignment.

The main objective of the following graph, is to identify and analyze which *Ectocarpus* peroxidases have a high number of hits in our transcriptomic data.

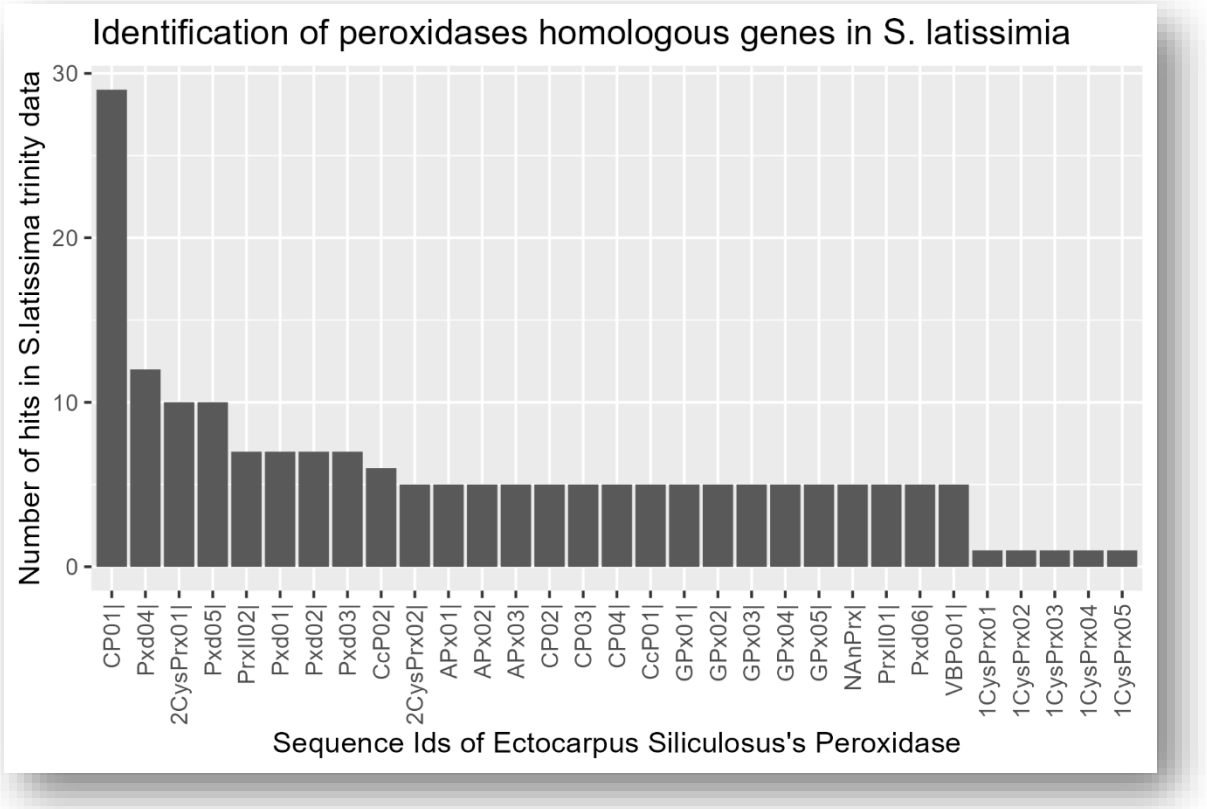


Figure 14 identification of E. *siliculosus* peroxidase gene in *S. latissima* genes

Gene Expression of peroxidases in S. latissima in different light

Principal Component Analysis (PCA) was used to study the effect of different light conditions on the gene expression patterns of *S. latissima*. The resulting PCA biplot (Figure 11) accurately summarises the variation found across the various experimental settings.

The PCA analysis found two main components that explain 67.9% of the overall variance in gene expression data. The principal axes of variation were revealed to be PC1, which contributed 47% of the variance, and PC2, which contributed 20.9%.

Examining the PCA biplot reveals a clear distinction between control conditions (C0, C1, C3, C9) and experimental light conditions (MED and MAX series). This distinction indicates significant changes in gene expression profiles between control samples and those exposed to different light intensities and durations. The evident segregation on the biplot indicates that light conditions have a significant impact on overall gene expression patterns in *S. latissimia*. The PCA results thus provide a comprehensive overview, illustrating how different light regimes influence gene expression and emphasising PCA's relevance in understanding complicated biological responses to environmental stimuli.

Data analysis of gene expression under varying light conditions reveals diverse patterns. Control samples (C0, C1, C3, and C9) had similar gene expression profiles, forming a coherent cluster on the left side of the plot. This implies that there is minimal variance under typical settings. Low light intensity conditions (MED series: MED1, MED3, and MED9) shift towards the center-right of the plot, demonstrating a gradual change in gene expression with longer duration (100 μmol $m^{-2}s^{-1}$). The position of MED1 closest to the control group indicates early reactions, whereas MED9 exhibits more dramatic alterations over longer exposure durations.

In contrast, high light intensity conditions (MAX series: MAX1, MAX3, MAX9) show a significant clustering towards the upper-right side of the plot. MAX1 shows significant changes in gene expression after just one day of exposure to high intensity light (250 μmol $m^{-2}s^{-1}$). The transition from MAX1 to MAX9 demonstrates further variation in gene expression over time in high-light situations. Principal Component Analysis identifies key axes of variation.

PC1 (47% variance) distinguishes between control and treated samples, highlighting the overall effect of light therapy on gene expression.

PC2 (20.9% variance) measures variances among treated samples, indicating how light intensity and duration contribute to variability in gene expression profiles.

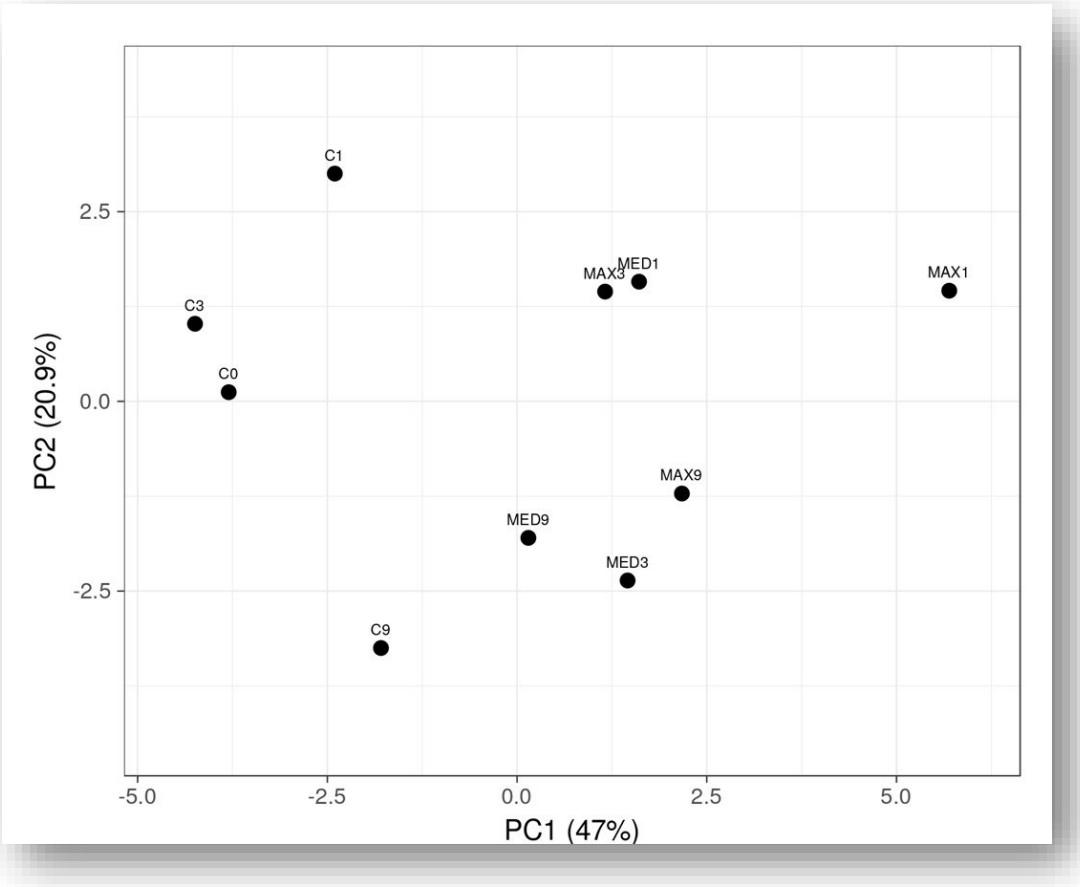


Figure 15 principal component analysis showing the top 21 peroxidase variable gene among the transcripts that aligned to assembly, across 10 sample groups (Co, C1, C3, C9, MED1, MED3, MED9, MAX1, MAX3, MAX9) in *S. latissimia* the following are abbreviations for control days: C0, C1, C3, and C9. The following are the sample numbers: MED1 = 100 µmol $m^{-2}s^{-1}$ light for 1 day, MED3 = 100 µmol $m^{-2}s^{-1}$ light for 3 days, MED9 = 100 µmol $m^{-2}s^{-1}$ light for 9 days, MAX1 = 250 µmol $m^{-2}s^{-1}$ light for 1 day, MAX3 = 250 µmol $m^{-2}s^{-1}$ light for 3 days, and MAX9 = 250 µmol $m^{-2}s^{-1}$ light for 9 days.

Gene expression regulation:

Figure 12 depicts a heatmap of peroxidase gene expression under diverse experimental settings, with rows representing individual peroxidase genes and columns representing different experimental setups. The colour gradient from blue to red represents relative expression levels, with blue representing lower expression and red indicating higher expression, and white denoting average expression.

Row hierarchical clustering displays groups of peroxidase genes with comparable expression patterns across circumstances, implying that experimental treatments produce particular responses. For example, genes such as 1CysPrx01 and GPx01 are highly expressed under C3 and C9 circumstances, showing divergent regulatory responses. Genes such as APx01 and Pxd01, on the other hand, have steady expression levels across all situations, implying a more consistent involvement under changing light conditions. Under low light circumstances (100 µmol $m^{-2}s^{-1}$, MED series), peroxidase genes like

NAnPrx and APx01 show higher expression for longer durations (from MED1 to MED9), showing a cumulative effect of prolonged exposure. Under high light settings (250 µmol $m^{-2}s^{-1}$, MAX series), genes such as 2CysPrx01 and GPx04 show increased expression over time, indicating a considerable impact of light intensity. Notably, CP02 expression is reduced under MAX3 and MAX9, indicating a possible light-induced downregulation mechanism.

A comparison of control conditions (C0, C1, C3, C9) and experimental settings (MED and MAX series) reveals dynamic variations in peroxidase gene expression influenced by light intensity and duration. The control circumstances serve as a baseline against which these changes are measured, highlighting the regulating effect of light conditions on peroxidase gene expression levels. Columns are hierarchically clustered to group circumstances with similar peroxidase gene expression profiles, revealing diverse patterns of reaction to different experimental treatments.

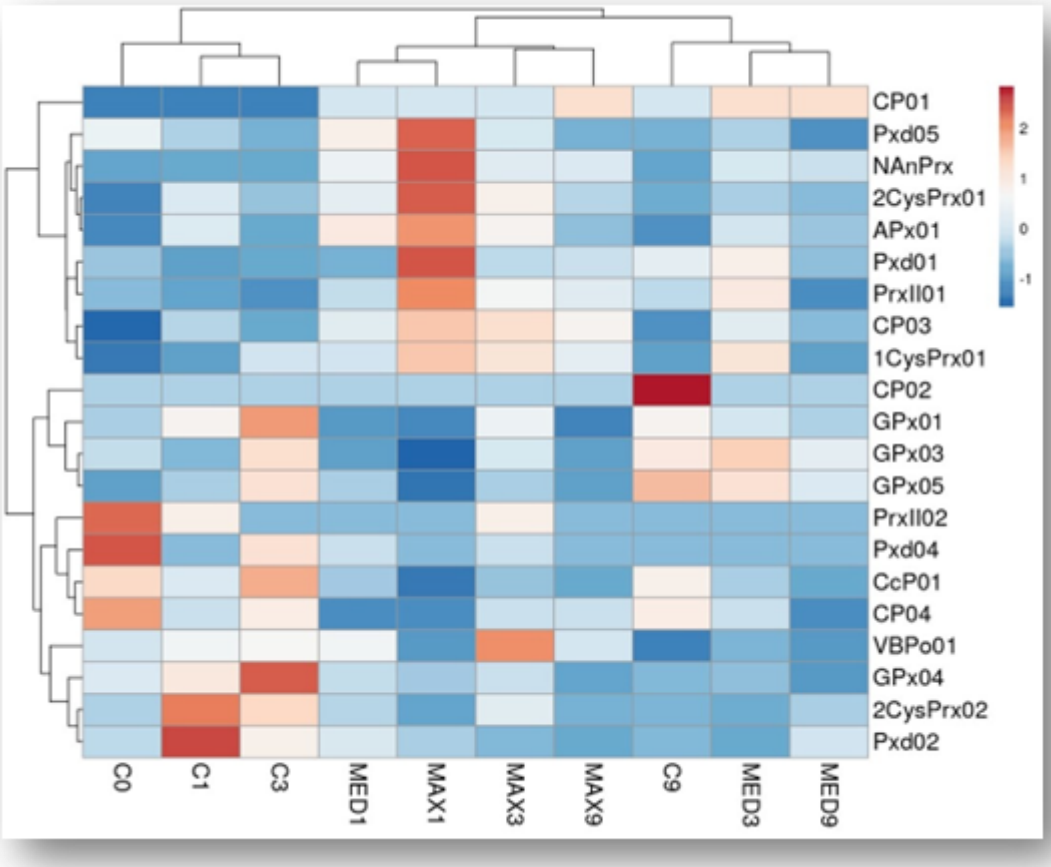


Figure 16 show the up and down regulation of peroxidases gene under different light intensity The scale from -1 (blue colour) to 2 (red colour) represents the degree of expression.

Overall statistical analysis of gene differential expression at medium light level vs control:

Genes	baseMean	log2FoldChange	lfcSE	Stat	Pvalue	Padj
2CysPrx02	31.87564308	-0.961926699	0.51881684	-1.854077634	0.06372805	0.223048189
2CysPrx01	3205.776643	0.402579735	0.396869446	1.014388332	0.31039748	0.592577025
GPx01	254.1234608	-0.460181122	0.265031713	-1.736324749	0.08250643	0.247519289
GPx04	187.2837767	-1.074276876	0.361519272	-2.971561845	0.00296289	0.06222073
Pxd01	11.0856508	0.497874561	0.9979279	0.49890835	0.617843952	0.763218999
Pxd02	6.466747553	-1.087525409	0.929453784	-1.170069376	0.241973051	0.564603786
Pxd04	1.327030213	-2.926293879	2.350587527	-1.244920194	0.213161087	0.559547854
Pxd05	7.008099825	0.043731118	0.671700327	0.065105101	0.94809031	0.948090319
Pxd06	0					
NAnPrx	34.31689873	0.926405635	0.402826826	2.299761522	0.021461734	0.112674106
VBPo01	178.3707305	-0.228475154	0.415388103	-0.55002816	0.58230005	0.763218999
GPx03	17.62728039	-0.049113834	0.451985188	-0.108662485	0.913470199	0.948090319
GPx05	8.159438696	-0.062652055	0.593826911	-0.105505583	0.91597464	0.948090319
CcP01	24.56980332	-1.177919526	0.435128849	-2.707059138	0.00678821	0.071276272
CcP02	0					
CP01	4.251725309	0.502311765	0.795953823	0.631081541	0.52798721	0.763218999
CP02	0.128208885	-0.736761098	3.816936256	-0.193024208	0.84694000	0.948090319
CP03	134.2342798	0.695658882	0.283732699	2.451810751	0.01421394	0.099497577
CP04	2.223244687	-1.213320235	1.133727613	-1.070204361	0.284527332	0.592577025
PrxII01	255.3835493	0.12452712	0.239414674	0.520131527	0.60297190	0.763218999
PrxII02	1.446062359	-0.813025438	1.35097324	-0.601807211	0.547302475	0.763218999
APx01	153.204399	0.961802959	0.457734092	2.101226403	0.035621097	0.149608606
1CysPrx01	9.518112614	0.335200793	0.549383043	0.610140406	0.54176880	0.763218999

Table7: Genes: Gene identifiers, baseMean: Average expression level across all samples, log2FoldChange: Logarithmic fold change in expression between MED and Control, lfcSE: Standard error of the log2 fold change, stat: Statistical test result, pvalue: p-value for differential expression, padj: Adjusted p-value for multiple testing correction.

We analysed the log2FoldChange values to determine which genes were upregulated or downregulated under MED (moderate) light levels based on the data provided. Fold changes provide a direct measure of how much a gene is upregulated or downregulated. Fold change is a common metric used in gene expression analysis to quantify the difference in expression levels of a gene between two conditions or samples. Positive numbers signified upregulation, whereas negative ones indicated

downregulation. We also looked at the statistical importance of these changes, as measured by the p-value. Typically, a p value less than 0.05 is considered statistically significant.

When discussing the results of gene expression analysis, it is important to emphasize the direction of regulation rather than solely focusing on the statistical significance of the expression differences. Given that the p-values are not statistically significant, we cannot make conclusive statements about the results. However, the data from log 2 fold change do provide indication of the direction of gene regulation.

The primary purpose of conducting a statistical analysis is to determine whether the observed differences in fold change are a result of the treatment or simply due to random variation. With a larger sample size, the p-values would likely reach statistical significance, strengthening the robustness of the results. Due to the current limited sample size, the focus remains on indicating the direction of regulation rather than asserting statistically significant upregulation or downregulation.

Significance: Although none of the padj values were less than 0.05, indicating that the changes were not statistically significant, the log2FoldChange values indicated the direction of regulation (up or down).
Upregulation: Genes with a positive log2FoldChange value were observed upregulated. *APx01, CP03,* and *NAnPrx* had positive log2FoldChange values, indicating that they were increased in gene expression under MED light conditions.
Downregulation: Genes with a negative log2FoldChange value were observed downregulated. *GPx04* and *CcP01* displayed negative log2FoldChange values, indicating that they were downregulated under MED light conditions.

Graphical explanation of gene expression at MED vs control condition

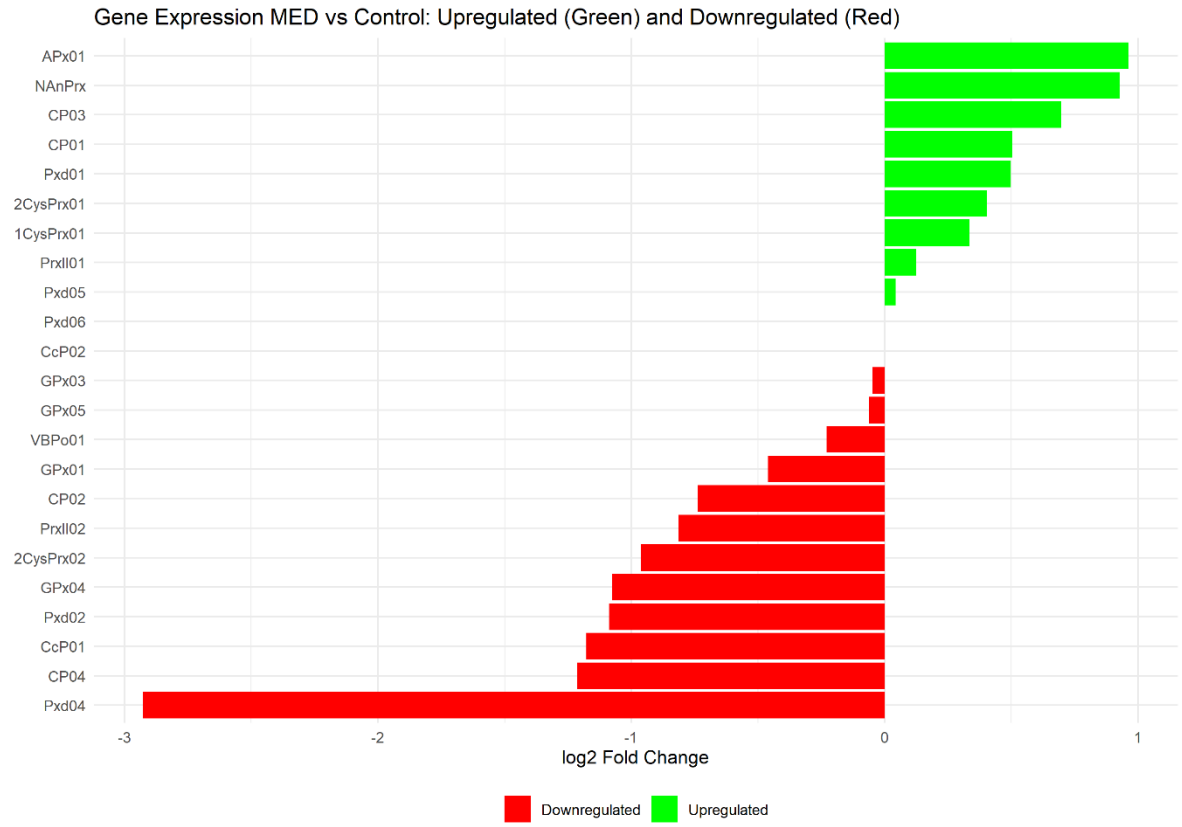


Figure 172: Y-Axis: Lists the gene names. X-Axis: Represents the log2 fold change in gene expression. Positive values (green bars) indicate upregulation in the MED condition, while negative values (red bars) indicate downregulation.

Overall statistical analysis of gene differential expression at maximum light level vs control:

Genes	baseMean	log2FoldChange	lfcSE	Stat	Pvalue	Padj
2CysPrx02	31.87564308	-1.042210484	0.51936059	-2.006718462	0.04477964	0.104485848
2CysPrx01	3205.776643	1.160421712	0.396731574	2.924954269	0.00344506	0.017533458
GPx01	254.1234608	-0.643213087	0.265601739	-2.421720166	0.015447241	0.046341722
GPx04	187.2837767	-1.012883789	0.360916882	-2.806418428	0.00500955	0.017533458
Pxd01	11.0856508	1.472568898	0.977847374	1.505929184	0.132085354	0.252162948
Pxd02	6.466747553	-2.209331707	1.010752154	-2.185829333	0.02882809	0.075673746
Pxd04	1.327030213	-2.983440357	2.350587527	-1.269231766	0.204358415	0.357627226
Pxd05	7.008099825	0.389461651	0.651323556	0.597954193	0.54987049	0.679251792
Pxd06	0					

NAnPrx	34.31689873	1.361886875	0.395778357	3.441034229	0.00057949	0.004056467
VBPo01	178.3707305	0.164021701	0.413856151	0.396325392	0.69186499	0.743486999
GPx03	17.62728039	-0.548855224	0.46706249	-1.175121608	0.23994606	0.38040759
GPx05	8.159438696	-0.596150594	0.62063591	-0.960548018	0.33677947	0.471491267
CcP01	24.56980332	-1.703935338	0.453302628	-3.758935492	0.00017063	0.001791697
CcP02	0					
CP01	4.251725309	0.345890347	0.802566878	0.430980092	0.66648284	0.743486999
CP02	0.128208885	-0.793907309	3.816936256	-0.207995957	0.835232124	0.835232124
CP03	134.2342798	1.257173769	0.280408207	4.483370089	7.34733E-06	0.000154294
CP04	2.223244687	-0.930285193	1.084022081	-0.858179191	0.39079352	0.512916501
PrxII01	255.3835493	0.367227985	0.238273686	1.541202435	0.123267525	0.252162948
PrxII02	1.446062359	-0.482738034	1.28925383	-0.374432111	0.70808285	0.743486999
APx01	153.204399	1.290484977	0.456660007	2.825920724	0.00471449	0.017533458
1CysPrx01	9.518112614	0.61095645	0.53515838	1.141636706	0.25360506	0.38040759

Table 8: Genes: Gene identifiers, baseMean: Average expression level across all samples, log2FoldChange: Logarithmic fold change in expression between MAX and Control, lfcSE: Standard error of the log2 fold change, stat: Statistical test result, pvalue: p-value for differential expression, padj: Adjusted p-value for multiple testing correction

Based on the analysis of the log2FoldChange values, we can observe specific patterns of gene regulation in the dataset. Several genes exhibit notable changes in their expression levels, either through upregulation or downregulation

Upregulated Genes:Among the genes that are upregulated, 2CysPrx01 shows a significant increase with a log2FoldChange of 1.16. Another gene, Pxd01, also displays upregulation with a log2FoldChange of 1.47. Additionally, NAnPrx is markedly upregulated with a log2FoldChange of 1.36, indicating a strong positive change in expression levels.

Downregulated Genes:Conversely, several genes are downregulated in this dataset. 2CysPrx02 is downregulated, showing a decrease in expression with a log2FoldChange of -1.04. GPx01 and GPx04 are also downregulated, with log2FoldChange values of -0.64 and -1.01, respectively

Graphical explanation of gene expression at Max vs control condition

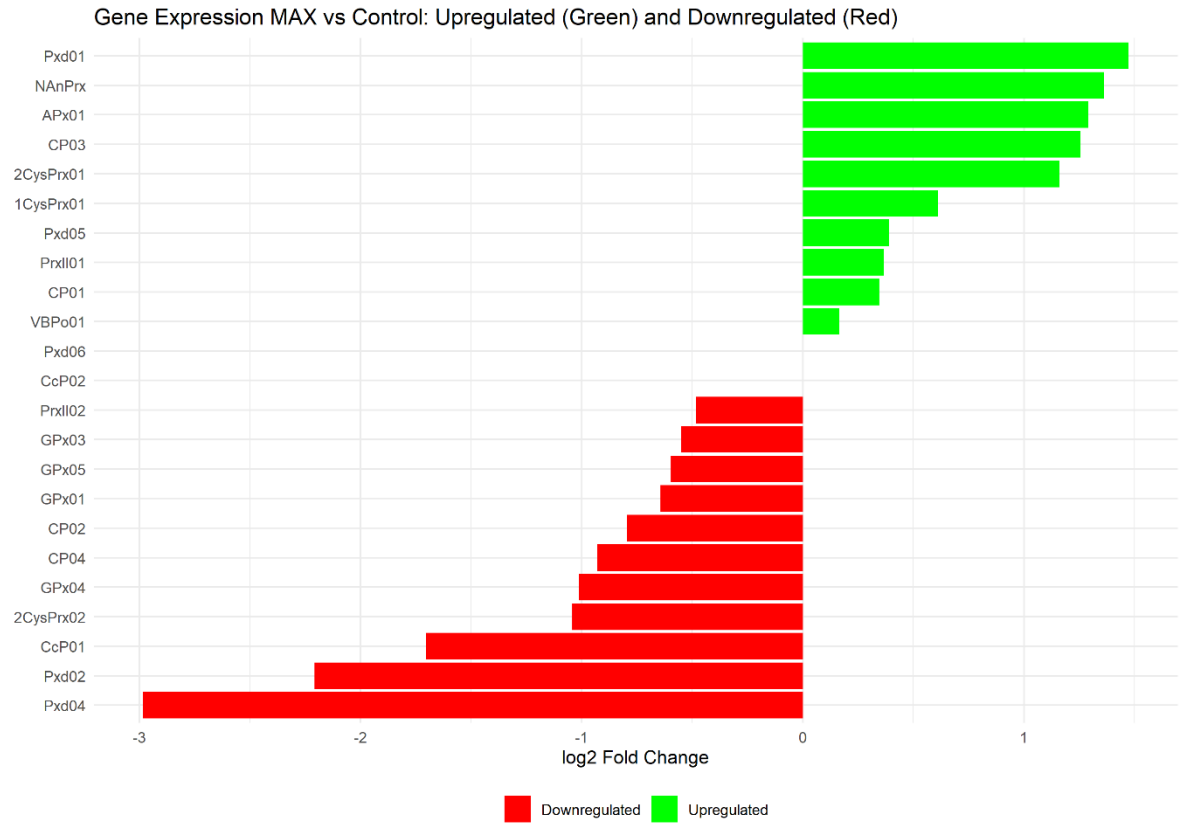


Figure 3: Y-Axis: Lists the gene names. X-Axis: Represents the log2 fold change in gene expression. Positive values (green bars) indicate upregulation in the MAX condition, while negative values (red bars) indicate downregulation.

Discussion:

Study 1: Cultivation of S. latissima gametophyte cultures with different nutrient solutions

Effect of nutrient solution on gametophyte growth:

In the first study, the growth analysis revealed several challenges: the overall analysis indicated that the growth conditions with ES were unfavorable as the growth trend was negative while 1/2Pes and F/2 showed no significant growth difference and growth rate was positive. In S. *latissima*, growth relies on essential nutrients like nitrogen, phosphorus, vitamins, iron and other trace metals.

They play crucial roles in cellular processes, including photosynthesis and DNA synthesis. ES medium had lack of such essential nutrients. Shortages in these nutrients can disrupt these processes, leading to negative growth. Various studies of growth showed that nitrogen is most likely the key limiting factor for growth in all treatments. It has been demonstrated that the addition of nutritional media improves growth capability (Forbord, 2020).

An imbalance in the nitrogen-to-phosphorus (N:P) ratio can have a major impact on microalgae development and composition (James j. Elser, 1990). The Redfield ratio for N:P is 16:1. When N:P deviate from this ratio, it can cause an imbalance that inhibits algae development. Higher N:P ratios then Redfield generally favour algae growth and biomass output.

The f/2 media we used here has a N:P ratio of around 24:1, while the 1/2PES (Provasoli Enrichment Solution) medium has a N:P ratio of about 18:1. Elevated nitrogen ratios can enhance algal growth and biomass output by supporting stronger cellular activities and protein synthesis. The Enriched Seawater (ES) medium has a N:P ratio of 8:1, indicating nitrogen restriction. Because phosphorus is more abundant than nitrogen, this lower N ratio may limit algae growth and biomass output. One of the previous studies showed f/2 medium (added nutrients) resulting in increasing levels of reproduction, gametophytes grew vegetatively in all cultures (Lüning & Neushul, 1978).

The relative growth rate and ANOVA results showed that growth of *S. latissima* differs due to nutrients solutions. The other suggestion could be the gametophyte cultures as some cultures may have genetic traits that allow them to thrive in one solution but not in another.

It should be mentioned that contamination is frequently a risk while growing gametophyte cells in open or partially enclosed systems. Microbes were unavoidably present during the whole culture process the decision not to add antibiotics was made because their presence may have unintended consequences on the physiology of the gametophytes, potentially altering their growth and development., even though every manipulation during the current culture including the cleaning of all tools and glassware, the treatment of the culture's saltwater, the addition of nourishment, and the replenishment of seawater was done with disinfection

Study 2: Transcriptome analysis of light-stressed S. latissima sporophyte with respect to peroxidases

Regulation of gene expression

The specific roles of these peroxidase genes, as defined in Tables 01 and 02 of the literature review, are described in detail.

Nanprx, cp03, and apx01 Upregulation

The genes *Nanprx, cp03*, and *apx01* showed upregulation according to log2fold change value under both MED and MAX light intensity conditions.

NAnPrx (Non-animal peroxidase) is a homodimeric enzyme expressed constitutively and present in the cytosol, peroxisomes, and mitochondria. It plays a crucial role in detoxifying reactive oxygen species (ROS), thus protecting the cell from oxidative damage. The upregulation of *NAnprx* under elevated

light intensities suggests an increased demand for ROS scavenging, which is a protective response to mitigate oxidative damage and maintain cellular homeostasis.

cp03 (Catalase class III peroxidase 3) is involved in various physiological processes, including response to biotic and abiotic stresses. Class III peroxidases, including cp03, function as fusion proteins with dual catalytic activity, operating both as a catalase and a peroxidase. The upregulation of cp03 indicates an enhanced defence mechanism against light-induced oxidative stress, suggesting that this peroxidase plays a significant role in protecting cellular components and ensuring plant resilience under increased light intensity.

apx01 (Ascorbate peroxidase 1) is crucial in the ascorbate-glutathione cycle, another important ROS detoxification pathway. Chloroplastic organisms contain *APx*, which shows significant selectivity for the electron donor ascorbate. The increased expression of apx01 under both light conditions further indicates an enhanced antioxidant defence mechanism. This response helps protect the photosynthetic apparatus and other cellular components from light-induced oxidative damage, ensuring optimal functioning and growth of the plant under stress conditions.

ccp01, cp04, and pxd04 **Downregulation**

Conversely, the genes *ccp01, cp04,* and *pxd04* were downregulated under both MED and MAX conditions.

ccp01 (Cytochrome c peroxidase 1) serves an important role in recycling hydrogen peroxide (H_2O_2) produced during aerobic respiration from cytochrome c oxidation in the mitochondrial intermembranous region. The downregulation of ccp01 might suggest a shift in the ROS detoxification burden away from the mitochondria, possibly towards cytosolic or chloroplast peroxidases like *Nanprx, cp03*, and *apx01*, which are upregulated. This could indicate a reorganization of the cellular antioxidant strategy, prioritizing enzymes that are more effective in the current oxidative stress context.

cp04 (Class III peroxidase 4) is another member of the Class III peroxidase family involved in various physiological processes, including lignin biosynthesis, suberization, and response to biotic and abiotic stresses. The downregulation of *cp04* might reflect a specific response to light-induced stress, where the energy and resources are redirected towards other ROS detoxification mechanisms that are more immediately beneficial under these conditions. Alternatively, this downregulation might be part of a regulatory mechanism to fine-tune the overall antioxidant response, preventing excessive peroxidase activity that could be detrimental.

pxd04 (Peroxidasin 4) is part of a superfamily of peroxidases that form covalent bonds with the apoprotein through their prosthetic heme group. Many members of this superfamily, including pxd04, are fusion proteins with non-peroxidase domains. The downregulation of pxd04 might indicate a strategic reduction in specific ROS-related processes, potentially to conserve energy and resources or to modulate the antioxidant defence balance in response to the specific oxidative stress induced by high light intensity.

Conclusion

The growth analysis of S. latissima revealed notable disparities among nutrient solutions, with 1/2Pes and F/2, characterized as nutrient-rich solutions, fostering positive growth rates, while the nutrient-deficient ES solution showed a significant negative growth trend. These insights underscore the critical role of balanced nutrient composition, particularly the N ratio, in promoting algal growth and inform future optimization strategies for cultivating S. latissima.

Differential regulation of antioxidant-related genes under varying light intensities highlights astrategic response in plants to mitigate oxidative stress. The upregulation of *Nanprx, cp03*, and *apx01* under both moderate (MED) and high (MAX) light conditions indicates an intensified demand for ROS detoxification mechanisms. These enzymes, localized across different cellular compartments, play crucial roles in scavenging reactive oxygen species (ROS) to maintain cellular homeostasis and protect cellular components from oxidative damage.

Conversely, the downregulation of *ccp01, cp04*, and *pxd04* suggests a reorganization of the antioxidant defense strategy in response to elevated light intensity. This modulation may reflect a shift in emphasis from certain peroxidases involved in specific ROS-related processes towards others that are more effective under light-induced oxidative stress conditions. Such adjustments likely optimize the balance between ROS production and scavenging, ensuring efficient utilization of cellular resources and maintaining overall plant resilience.

Exploring the precise regulatory mechanisms governing the differential expression of antioxidant genes in *S. latissima* under varying light intensities will deepen insights into its adaptive responses to environmental stress. Utilizing techniques such as transcriptomics and proteomics will elucidate the complex network of gene interactions and pathways involved. Comparative studies across different environmental conditions and *S. latissima* varieties can validate these findings, revealing species-specific adaptations that inform strategies for enhancing resilience in *S. latissima* cultivation. Functional validation through genetic manipulation studies will confirm the roles of identified genes in oxidative stress tolerance, potentially identifying targets for genetic improvement.

There are intriguing avenues for further investigation based on the data in this study. Creating new de novo assemblies and comparing them to existing ones could provide useful information about the transcriptome landscape. Furthermore, gene enrichment and KEGG pathway analysis have the potential to improve our understanding of the complex transcriptome mechanisms reported in this study.

These studies would not only improve present findings, but would also add to a more thorough and nuanced understanding of the underlying molecular pathways.

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Appendix

Relative growth Rate

```
library(readxl)
df <- read_excel("C:/Users/RABYA ASHFAQ/Desktop/Master Thesis/Rfiles/results.xlsx",
                 sheet = "Sheet3")
View(df)
#Relative Growth Rate = (ln(Wt2/Wt1)) / (t2 - t1)
library(dplyr)
# Assuming data is already sorted by time
df <- df %>%
  group_by(Cultures, Solutions) %>%
  mutate(RelativeGrowthRate = (log(W_2) - log(W_1)) / (T_1 - T_0))
library(ggplot2)
ggplot(df, aes(x = T_1, y = RelativeGrowthRate, color = Cultures)) +
  geom_bar(stat = "identity", position = "dodge") +
  ggthemes::theme_economist() +
```

```
  facet_wrap(~Solutions, scales = "free_y") +
  labs(x = "Time", y = "Relative Growth Rate", title = "Relative Growth Rate of S.Latisimia")
theme(plot.title = element_text(margin = margin(b = 12))) +
  theme(axis.title.y = element_text(margin = margin(r = 10)),
        axis.title.x = element_text(margin = margin(t = 6)),
        plot.title = element_text(size=12, face = "bold.italic"))
```

cumulative growth rate

```
Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))
# Define the growth rates
growth_rates <- c(0.775, 1.0616, 1.307)
# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]
# Loop through the remaining days and calculate cumulative growth
for (day in 2:50) {
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }}

# Print the resulting data frame



colnames(cumulative_growth) <- c("Day", "20SpN4-F/2")
A<- cumulative_growth
# Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))
# Define the growth rates
growth_rates <- c(0.57, 0.688333333, 0.711)
# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]
# Loop through the remaining days and calculate cumulative growth
for (day in 2:50) {
```

```
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }
}

# Print the resulting data frame
colnames(cumulative_growth) <- c("Day", "19SpN4-F/2")
B<- cumulative_growth
# Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))
# Define the growth rates
growth_rates <- c(1.235,            0.895,   1.05)
# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]
# Loop through the remaining days and calculate cumulative growth
for (day in 2:50) {
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }}
# Print the resulting data frame
colnames(cumulative_growth) <- c("Day", "17SpN2-F/2")
C<- cumulative_growth
# Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))
# Define the growth rates
growth_rates <- c(0.92, 0.815, 0.914)
# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]
# Loop through the remaining days and calculate cumulative growth
```

```
for (day in 2:50) {
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }}
# Print the resulting data frame
colnames(cumulative_growth) <- c("Day", "20SpN4-1/2PES")
D<- cumulative_growth
# Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))
# Define the growth rates
growth_rates <- c(0.135,            1.215, 1.309)
# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]
# Loop through the remaining days and calculate cumulative growth
for (day in 2:50) {
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }}
# Print the resulting data frame
colnames(cumulative_growth) <- c("Day", "19SpN4-1/2PES")
E<- cumulative_growth
# Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))
# Define the growth rates
growth_rates <- c(1.315, 0.78, 0.731)
# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]
# Loop through the remaining days and calculate cumulative growth
for (day in 2:50) {
```

```
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }
}
# Print the resulting data frame
colnames(cumulative_growth) <- c("Day", "17SpN2-1/2PES")
Ff<- cumulative_growth
# Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))
# Define the growth rates
growth_rates <- c(0.19, 0.146666667, 0.05)
# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]

# Loop through the remaining days and calculate cumulative growth
for (day in 2:50) {
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }
}
# Print the resulting data frame
colnames(cumulative_growth) <- c("Day", "20SpN4-ES")
G<- cumulative_growth
# Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))
# Define the growth rates
growth_rates <- c(-0.7, 0.336666667, 0.438)
# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]
```

```
# Loop through the remaining days and calculate cumulative growth
for (day in 2:50) {
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }
}
# Print the resulting data frame
colnames(cumulative_growth) <- c("Day", "19SpN4-ES")
H<- cumulative_growth
# Create an empty data frame to store the results
cumulative_growth <- data.frame(Day = 1:50, Growth = numeric(50))

# Define the growth rates
growth_rates <- c(-1.17,-0.328333333,   -0.31)

# Initialize the cumulative growth
cumulative_growth$Growth[1] <- growth_rates[1]

# Loop through the remaining days and calculate cumulative growth
for (day in 2:50) {
  if (day <= 10) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[1]
  } else if (day <= 30) {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[2]
  } else {
    cumulative_growth$Growth[day] <- cumulative_growth$Growth[day - 1] + growth_rates[3]
  }
}

# Print the resulting data frame
colnames(cumulative_growth) <- c("Day", "17SpN2-ES")
I<- cumulative_growth
cumulative<- merge(A,B,by = "Day")
```

```
cumulative<- merge(cumulative, C, by= "Day")
cumulative<- merge(cumulative, D, by= "Day")
cumulative<- merge(cumulative, E, by= "Day")
cumulative<- merge(cumulative, Ff, by= "Day")
cumulative<- merge(cumulative, G, by= "Day")
cumulative<- merge(cumulative, H, by= "Day")
cumulative<- merge(cumulative, I, by= "Day")

library(ggplot2)
#install.packages("reshape2")
library("reshape2")
new<- melt(cumulative, id = "Day")
library(tidyverse)
#new %>%
 # ggplot(aes(Day, value)) +
  #geom_point(aes(color=variable)) +
  #facet_grid(value ~ variable)+
  #stat_smooth(method = "lm",
   #          formula = y ~ x,
    #         geom = "smooth")
aa<- unlist(strsplit(as.character(new$variable), "-"))
data <- data.frame(culture = aa[seq(1, length(aa), by = 2)],
                   solution = aa[seq(2, length(aa), by = 2)])
cumm<- cbind(new, data)
cumm <- cumm[, c(1,4,5,3)]
library(tidyverse)
library(dplyr)
library(ggplot2)
H <-cumm %>%
  ggplot(aes(Day, value)) +
  geom_point(aes(color=culture)) +
  facet_grid(culture ~ solution)+
   labs(title = 'Cumulative Growth Rate', y = 'Absolute weight' , x = 'Number of Days')
###run whole formula together
ggsave("output_plotC.tiff", plot = H , device = "tiff", width = 6, height = 4, dpi = 300)
```

ANOVA

```
# Load necessary libraries
library(readxl)
library(tidyverse)
library(ggpubr)
library(rstatix)

# Read the data
df <- read_excel("C:/Users/Rbyaa Ashf/Desktop/new_results.xlsx", sheet = "Sheet8")

# Data preparation
df <- df %>%
  gather(key = 'time', value = 'growth', G10, G30, G50)
df$time <- as.factor(df$time)

# Sample data for checking
df %>% sample_n_by(Solutions, time, size = 1)

# Summary statistics
df %>% group_by(Solutions, time) %>%
  get_summary_stats(growth, type = 'mean_sd')

# Checking outliers
df %>% group_by(Solutions, time) %>%
  identify_outliers(growth)

# Normality test
df %>% group_by(Solutions, time) %>%
  summarise(shapiro_p_value = shapiro.test(growth)$p.value)

# Dot plot
dot_plot <- ggdotplot(df, x = 'time', y = 'growth',
                      color = 'Solutions', palette = 'jco',
                      add = 'mean_sd',
                      position = position_dodge(0.8)) +
  theme_bw() +
  labs(title = "Dot Plot of Growth Over Time by Solutions",
```

```
      x = "Time",
      y = "Growth") +
  theme(legend.position = "right")

print(dot_plot)

# QQ plot
ggqqplot(df, 'growth', ggtheme = theme_bw()) +
  facet_grid(time ~ Solutions, labeller = 'label_both')

# ANOVA computation
res.aov <- anova_test(df, dv = growth, wid = Strains,
                      within = c(Solutions, time))
get_anova_table(res.aov)
```

Pie Chart

```
library(tidyverse)
library(dplyr)
library(readxl)
library(ggplot2)
library(readxl)
df <- read_excel("C:/Users/RABYA ASHFAQ/Desktop/Master Thesis/ectocarpus peroxidases
27.xlsx")
View(df)
library(readxl)
df <- read_excel("C:/Users/RABYA ASHFAQ/Desktop/Master Thesis/Rfiles/ectocarpus peroxidases
27.xlsx")
View(df)
df$gene <- as.factor(df$gene)
df$peroxidase <- as.factor(df$peroxidase)
###############
library(tidyverse)
df <- df %>%
  group_by(peroxidase) %>% # Variable to be transformed
  count() %>%
  ungroup() %>%
  mutate(perc = `n` / sum(`n`)) %>%
  arrange(perc) %>%
```

```
  mutate(labels = scales::percent(perc))
E <- ggplot(df, aes(x = "", y = perc, fill = peroxidase)) +
  geom_col(color = "black") +
  geom_label(aes(label = labels),
             position = position_stack(vjust = 0.5),
             show.legend = FALSE) +
  coord_polar(theta = "y") +
  ggtitle("Percentages of Heam and Non-Heam peroxidases group")
# Save the plot
ggsave("output_plotE.tiff", plot = E, device = "tiff", width = 6, height = 4, dpi = 300)
```

Identification of homologous peroxidase gene:

```
library(readxl)
df <- read_excel("C:/Users/RABYA ASHFAQ/Desktop/Master
Thesis/Rfiles/blastn_kelp_peroxidases.xlsx",                    sheet = "Sheet1")
View(df)
df$pident <- as.numeric(as.character(df$pident))
count_df <- df %>%
  group_by(qseqid) %>%
  summarise(count = n())
count_df$qseqid <- factor(count_df$qseqid, levels = count_df$qseqid[order(-count_df$count)])
# Create the plot
p <- ggplot(count_df, aes(x = qseqid, y = count)) +
  geom_bar(stat = "identity") +
  labs(x = "Sequence Ids of Ectocarpus Siliculosus's Peroxidase", y = "Number of hits in S.latissima
trinity data") +
  ggtitle("Identification of peroxidases homologous genes in S. latissimia") +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1))
# Save the plot
ggsave("output_plotD.tiff", plot = p, device = "tiff", width = 6, height = 4, dpi = 300)
```

Deseq2

```
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install("DESeq2")
# Load DESeq2 library
library("DESeq2")


# Load the data
```

```
counts <- read.csv("C:/Users/Rbyaa Ashf/Desktop/Master Thesis/counts_matrix.csv", row.names = 1)
colData <- read.csv("C:/Users/Rbyaa Ashf/Desktop/Master Thesis/col_data.csv", row.names = 1)

# Ensure conditions are treated as factors
colData$condition <- factor(colData$condition, levels = c("Control", "Medium", "Maximum"))

# Create DESeqDataSet
dds <- DESeqDataSetFromMatrix(countData = counts, colData = colData, design = ~ condition)

# Run the differential expression analysis
dds <- DESeq(dds)

# Extract results for each condition comparison
resultsNames(dds)  # to see the different comparisons available
res_medium_vs_control <- results(dds, contrast = c("condition", "Medium", "Control"))
res_maximum_vs_control <- results(dds, contrast = c("condition", "Maximum", "Control"))
# Save results to CSV files
write.csv(as.data.frame(res_medium_vs_control), file = "C:/Users/Rbyaa Ashf/Desktop/Master
Thesis/differential_expression_results_medium_vs_control.csv")
write.csv(as.data.frame(res_maximum_vs_control), file = "C:/Users/Rbyaa Ashf/Desktop/Master
Thesis/differential_expression_results_maximum_vs_control.csv")
# Save results to xlsx
library(writexl)
write_xlsx(as.data.frame(res_maximum_vs_control),
'differential_expression_results_maximum_vs_control.xlsx')
write_xlsx(as.data.frame(res_medium_vs_control),
'differential_expression_results_medium_vs_control.xlsx')
# Print summary of results
summary(res_medium_vs_control)
summary(res_maximum_vs_control)
```

Differential expression MED VS CONTROL

```
# Load necessary libraries
library(ggplot2)

# Create the data frame
```

```
data <- data.frame( C:/Users/Rbyaa Ashf/Desktop/Master
Thesis/differential_expression_results_medium_vs_control.csv)
)

# Create the bar plot
plot <- ggplot(data, aes(x = reorder(Genes, log2FoldChange), y = log2FoldChange, fill =
log2FoldChange > 0)) +
  geom_bar(stat = "identity") +
  scale_fill_manual(values = c("red", "green"), labels = c("Downregulated", "Upregulated")) +
  coord_flip() +
  labs(title = "Gene Expression MED vs Control: Upregulated (Green) and Downregulated (Red)",
       x = "",
       y = "log2 Fold Change") +
  theme_minimal() +
  theme(legend.title = element_blank(),
        legend.position = "bottom")

# Save the plot as a TIFF file
tiff("gene_expression MED vs Control_plot.tiff", width = 10, height = 7, units = 'in', res = 300)
print(plot)
dev.off()
```