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# The role of manganese in light stress-induced tipburn and growth of lettuce *(Lactuca sativa L."Frillice")*

Gifty kodua
Plant Sciences- Plant production systems

## Preface

To the Glory of God, this master thesis was conducted as part of a research project “Control of tipburn for increased production of Frillice´ lettuce. The project was funded by the Norwegian Research Council (NRC) and Grofondet in cooperation with the Norwegian University of Life Sciences (NMBU).

The research project aims at getting deeper insight into the problem of tipburn in frillice lettuce produced in a greenhouse by performing experiments. The experiments focused on developing a better environment for frillice lettuce to help reduce the quantity and severity of tipburn to avoid losses.

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Ås, 15.08.2022

Gifty Kodua

## Summary

Consumption of vegetables like lettuce is on the increase worldwide because of its health-related benefits. However, tipburn remains one of the major problems of lettuce, especially in a controlled environment. Tipburn is a physiological disorder seen as necrosis at the apex of leaf margins. The problem reduces the quality of the final product leading to economic losses. The cause of the problem has been attributed to insufficient calcium supplied to the fast-expanding leaves. Several abiotic factors like light, temperature and relative air humidity among others have been proven to influence this problem. Studying the interaction between cultivated lettuce and the environment will provide information and an understanding of the plant's biological system. This in turn will help to develop more effective strategies for improving the plants' yield, quality, and sustainability.

Manganese is an essential microelement for plant growth and development. It is involved in several metabolic processes, mainly in photosynthesis and as an enzyme antioxidant-cofactor. Nonetheless, an excess of this micronutrient can be toxic for plants. In this thesis, the role of extra manganese (111ppm) in the nutrient solution and foliar spray (15ml of Mn/1L $H_2O$ three times during the experiment) in light stress-induced tipburn occurrence and the severity of lettuce (Lactuca Sativa L. 'Frillice') was investigated. The effect on the plants' growth, the content of other cations, and antioxidant capacity were also investigated.

Light spectral distribution and intensity are important in plants' growth and development and differ from lamp to lamp. In the experiments, High pressure sodium (HPS) and light emitting

diode (LED) lamps were used in plants treated with extra Mn in the nutrient solution. The research focused on manipulating the irradiance, from low (150 µmol $m^{-2}$ $s^{-1}$) to high (300 µmol $m^{-2}$ $s^{-1}$) in HPS and low (134 µmol $m^{-2}$ $s^{-1}$) and high (206 µmol $m^{-2}$ $s^{-1}$) in LED of plants growing with elevated Mn in the nutrient solution. The effect of foliar application was tested on plants in high (270 µmol $m^{-2}$ $s^{-1}$) and low (135 µmol $m^{-2}$ $s^{-1}$) light with LED.

Growth assessment was performed on plants treated with extra Mn in the nutrient solution and plants treated with foliar spray. Foliar application of extra Mn did not affect either plant growth or tipburn severity (both inner tipburn and outer tipburn) either in low light or high light with LED. Nutrient content analysis was performed on inner leaves, edge of old leaves, and inner part of old leaves for selected plants treated with extra Mn in the nutrient solution. Content of Mn, Ca, Mg, K, and Zn was measured to find the effect of treating plants with extra Mn in the nutrient solution on these elements. An analysis of antioxidant capacity (FRAP) was also performed on young leaves of plants from high (206 µmol $m^{-2}$ $s^{-1}$) and low (134 µmol $m^{-2}$ $s^{-1}$) in LED grown with extra Mn in the nutrient solution to find out if there exists any relationship between Mn accumulation, antioxidant capacity (FRAP) and tipburn occurrence.

Additional Mn in the nutrient solution led to a higher concentration of this element in all parts of frillice lettuce analyzed. However, the content of inner leaves was much lower than the edge of outer old leaves. The effect of adding Mn on concentrations of Ca, K, and Mg was not significant under HPS but significantly reduced Mg was found with LED. Zn in most cases was below detection level.

Adding more Mn led to the nutrient solution increased Mn content in the inner leaves and led to less inner tipburn. The higher accumulation of Mn in outer leaves did not reduce the incidence of outer tipburn. Hence, it is possible to reduce the inner tipburn by increasing the Mn content of inner leaves but the outer tipburn will probably increase. Outer tipburn severity was dependent on lamp type and irradiance where severity was high in plants grown in high light with LED lamps. Hence, to reduce the outer tipburn, plants have to be grown with a rather low irradiance, LED or HPS lamps.

A significant increase in fresh weight and dry weight of plants treated with extra Mn in the nutrient solution was recorded in high light with HPS. Under LED, low light did not significantly

affect fresh weight whilst high light significantly reduced it. Antioxidant capacity was high in plants exposed to LED in high light but did not change in response to Mn content and was not correlated with tipburn incidence. This thesis shows that Mn has a role in the development of tipburn, but the effect seems to vary with tissue type (young inner leaves versus old outer leaves) and lamp type. Small effects were found on growth and development.

## Abbreviations

PPFD = Photosynthetic Photon Flux Density

HPS = High Pressure Sodium

LED = Light Emitting Diodes

FRAP = Ferric Reducing Antioxidant Power

RH = Relative air humidity

SOD = Superoxide dismutase

ROS = Reactive Oxygen Species

EC = Electric conductivity

W = Watts

C = Celsius

Mn = Manganese

Ca = Calcium

Mg = Magnesium

K = Potassium

FW = Fresh weight

DW = Dry weight

WC = Water content

PAR = Photosynthetic Active Radiation

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# 1 Introduction

Increased consumption of vegetables has been associated with reduced risks of chronic diseases like cancer, cardiovascular disease, and age-related functional decline. Lettuce is a good source of fiber, iron, folate, and vitamin C. It also supplies large amounts of antioxidants and polyphenols (Serafini et al., 2002; Kim et al., 2016).

For some years now, hydroponics has been successfully utilized for lettuce cultivation. This has contributed to the diversification of production methods and the reduction of water usage and excessive fertilization. Greenhouse production using the nutrient film technique (NFT) is common in Norway. One of the major limitations of greenhouse lettuce production is tipburn (Birlanga et al., 2021).

Tipburn is defined as localized necrosis found on the distal margins of rapidly expanding leaves (Figure 1). It is a serious problem in controlled lettuce production reducing the quality and shelf

life of fresh lettuce. This results in severe economic losses for growers. The condition has been associated with insufficient transport of calcium to the fast-growing areas of the leaves rather than uptake by roots or adequate supply in the nutrient solution (Barta & Tibbitts, 1986). Although calcium deficiency has been considered the main factor causing tip burn in lettuce, it is influenced by environmental factors, such as light intensity, air temperature, and soil conditions. The susceptibility of plants to tipburn is genetically determined (Saure, 1998). An optimized plant environment with high light results in higher growth rates, therefore lettuce production in controlled environments is particularly sensitive to tipburn (Frantz et al., 2004). Frillice is an important cultivar among Norwegian growers and consumers prefer this cultivar because of its sweet taste. However, tipburn remains one of the major problems in this cultivar confronting the growers.

Manganese (Mn) is an essential micronutrient involved in redox reactions as a cofactor for many enzymes including the Mn- superoxide dismutase (Mn-SOD) which protects plants against oxidative stress (González et al., 1998). It is also part of the water-splitting system that provides electrons to photosystem II (PS II) (Alejandro et al., 2020). Mn is easily accumulated in aerial parts of leafy vegetables (Kleiber, 2014). As a fast ligand exchanger, it can easily replace other divalent metal ions, like Mg, Ca, Fe, Co, Cu, and Zn. However, excess of Mn can lead to increased production of ROS leading to oxidative damage. Excess can also alter processes like enzymatic activity, uptake, redistribution, and the use of other nutrients like Ca, Fe, Mg, N, and P. This can lead to changes in productive responses of agricultural crops (Lavres Junior et al., 2010). Toxicity symptoms include chlorosis, necrosis, crinkled leaves, and stunted growth. A plant's response to extra Mn is affected by factors like nutrient balance, temperature, light intensity, and genotype.

This thesis seeks to investigate the role of extra Mn (111ppm) in light stress-induced tipburn (both outer and inner tipburn) occurrence and severity of frillice lettuce. Since the severity of Mn toxicity symptoms and tipburn often depends on the light intensity, the experimental setup focused on growing plants under different irradiance. This will help to assess how Mn and other cations accumulate in inner leaves, inner part of old leaves, and edge of old leaves in varying irradiance and find if it correlates with tipburn occurrence and severity in frillice lettuce. High

pressure sodium (HPS) is a common lamp used among Norwegian commercial greenhouse growers whilst light emitting diodes (LEDs) is a new technology that makes it possible to choose specific spectra for plant's requirement. These two lamp types were therefore deployed in this study.

Extra Mn is known to induce oxidative stress, which in turn triggers the production of antioxidants. Analysis of antioxidant capacity was also performed on inner leaves of plants in both low and high light with LED.

*Figure 1: Tipburn of lettuce*

## 1.1 Objectives

The main objective of this thesis was to investigate the role/effect of manganese (Mn) on tipburn occurrence and severity in lettuce cv. Frillice. The sub-goals were to:

1. To test if additional Mn in nutrient solution or Mn foliar spray will affect growth and tipburn development in frillice lettuce under different light intensities.
2. To test if extra Mn in nutrient solution will affect tipburn development in frillice lettuce either with HPS or LED as a light source
3. To assess the effect of extra Mn in nutrient solution on the accumulation of Ca, K, Mg, Mn, and Zn in frillice lettuce grown under different light intensities and lamp types (HPS and LED).
4. To test if extra Mn in nutrient solution will increase antioxidant capacity in frillice lettuce grown with LED light.

# 2 Literature review

## 2.1 Lettuce *(Lactuca sativa L. 'Frillice')*

The name Lettuce is linked to the genus Lactuca. Lactuca sativa was a roadside weed that later became cultivated lettuce. It is believed to be indigenous to the southern shores of the Mediterranean Basin from Egypt eastward into Asia Minor where it was first domesticated. From there, lettuce cultivation spread into Rome, Greece, and other parts of the world after Columbus' first voyage in 1494 (Whitaker, 1969).

Lettuce is a cool-season plant that belongs to the *Asteraceae* family. It is mainly cultivated in temperate and subtropical regions. It is a self-pollinated plant with a deep taproot and horizontal lateral roots near the soil surface for water and nutrient absorption. The leaves are arranged in a rosette with a shortened stem. Leaves vary in color, shape, surface, margin, and texture among types and forms (Mou, 2008).

A survey by NORBAGREEN showed that green salad was the third most popular vegetable in Sweden and Åland, fourth in Finland and Denmark, and sixth in Norway. Most lettuce varieties seen in most Scandinavian food shops today derive from the four genera Lactuca (e.g., iceberg lettuce, oakleaf lettuce), Chicorium (e.g., frillice lettuce), Eruca (e.g., rocket), and Valerianella (e.g., manche) (Johansson et al., 2007).

Generally, six types are recognized based on leaf shape, size and texture, head formation, and stem type: (i) crisphead/iceberg; it produces a spherical firm head with bright/dull green outer leaves which is brittle and crispy with a mild taste (ii) butterhead/cabbage lettuce; produces small and less compact head than crisphead lettuce. Leaves are broad, crumpled, thin, and tender with oily texture; (iii) romaine; has upright stature and forms a loaf-shaped head after the rosette stage. Leaves are coarse and crispy with broad midveins. (iv) Leaf lettuce; it varies in leaf size, shape, color, and texture. It has a stronger taste than the crisphead type. (v) stem/ asparagus lettuce; grown for its thick erect stem. (vi) Latin; has upright stature like romaine lettuce. Leaves are shorter and less crispy (Mou, 2008). Iceberg is the most popular lettuce type in Scandinavian (Mou, 2008).

The cultivar Frillice is obtained by crossing leaf lettuce endive, and iceberg lettuce. It is very crispy, has a sweet taste, and is resistant to bolting (Knoop, 2019). It is a popular lettuce type in Norway and is produced in greenhouses, plastic tunnels, and open fields.

Lettuce grows well in moderate temperatures ranging from 17ºC-28ºC with an optimum day temperature of 18ºC and more than 15ºC at night (Ah-Chiou et al., 2015; Knoop, 2019). It is often grown in low light, (often the maximum is 400 μmol $m^{-2}$ $s^{-1}$) to minimize tipburn and improve quality (Saure, 1998).

Today, breeding programs have helped with cultivars' resistance to diseases and insects, increased production, and development of new types with attributes like less bitterness and deterioration, resistance to tipburn, and superior flavor (Sulaiman et al., 2011).

## 2.2 Nutritional composition and use of lettuce

Increased consumption of vegetables is known to reduce the risk of diseases like cancer, heart disease, and age-related functional decline. Lettuce is among the most popular leafy vegetable among the salad vegetable crops due to its health effect (Llorach et al., 2008). It is usually used fresh with other vegetables like tomato, carrot, and cucumber or served alone. They supply large amounts of antioxidants and polyphenols as well as fibers (Serafini et al., 2002).

Quality indicators include the color and texture of a leaf, the composition of various nutrients, and the chemicals it contains. This differs among varieties. These qualities are affected by growing conditions during production and the plants' genotype. However, growing conditions can be manipulated to get desired qualities in a controlled environment (Pérez-López et al., 2013).

Lettuce contains compounds like phenolics, vitamin C, folates, carotenoids, and chlorophylls which are essential for good health. These compounds have an antioxidant capacity that can protect the body against various diseases. According to Sulaiman et al. (2011), phenolics contribute to antioxidant capacity in plants because of their ability to donate hydrogen atoms to free radicals. Also, they have an ideal structure for scavenging free radicals. Carotenoids (β-carotene, α-carotene, and β-cryptoxanthin) and pro-vitamin A have antioxidants capacity and increase activity against free radicals when mixed with other antioxidants like vitamin E. Vitamin C takes part in many biochemical mechanisms and reduces oxidative free radicals both in vivo and in vitro (Duarte & Lunec, 2005). Folates have been compared with the activities of vitamin C and D which act as antioxidants (López et al., 2014).

Lettuce is also low in calories and provides the body with dietary fiber which aids the proper functioning of the digestive system. It is also rich in minerals like calcium and iron. The composition and quantity of phytochemicals vary in different cultivars. It is therefore important to consider them when choosing a cultivar during production (Kim et al., 2016). Other uses include making cigarettes without nicotine from the leaves, edible oil from seeds of primitive types, and sedative from dried latex in the stem of, Lactuca virosa L. (Ryder, 1986).

On the other hand, leafy vegetables like lettuce contain nitrate which can have adverse effects on human health when taken in large quantities. Low nitrate content in vegetables is very important for human health since nitrate can be reduced to nitrite which then combines with secondary

amines to increase the risk of gastrointestinal cancer (Hord et al., 2009). Variation in nitrate content in lettuce exists among cultivars. This variation is caused by environmental conditions, nitrogen supply, and genetics (Escobar-Gutierrez et al., 2002). In the greenhouse, low temperatures and light may lead to the accumulation of high levels of nitrate. This can be resolved by using supplementary lighting (Knoop, 2019). In the work of Zhou et al. (2011), increased irradiance from 50 to 200 µmol $m^{-2}$ $s^{-1}$ decreased the nitrate content of indoor cultured lettuce. Other salts or NaCl can be added to the nutrient solution to reduce the nitrate content.

## 2.3 Lettuce production in a controlled environment

Growing in a controlled environment has become popular and necessary worldwide because of climate change, increasing drylands, reduction in freshwater supply, and population growth relative to arable land (Fedoroff, 2015). In Norway, short growing seasons, and lack of natural light in most months of the year make it extremely necessary to produce in a controlled environment. In a controlled environment like the greenhouse, there is increased crop protection, efficient water and fertilizer usage, and higher productivity (Stanghellini, 2019). Production in a controlled environment also ensures uniform and predictable growth and development, high crop value per unit of production area, and a short production period (Dreesen & Langhans, 1992). According to Barbosa et al. (2015), greenhouse production gives a higher yield and is more efficient in terms of water usage when compared with conventional farming. Meanwhile, it requires high energy for supplementary lighting and to heat the greenhouse to the required temperature to achieve higher yields. Ensuring optimal environmental conditions in a controlled environment maximizes lettuce light use efficiency and growth (Ahmed et al., 2020).

Lettuce is among leafy vegetables produced in controlled environments with artificial lighting making year-round production possible. Hydroponic is a popular system used among Norwegian frillice lettuce growers specifically the nutrient film technique (NFT). This system among others like the floating systems or closed hydroponic methods has been successfully used for lettuce production.

In the NFT system, plant roots grow in gutters with a nutrient solution (water and nutrients) (Bernardes, 1997) which can be recirculated for plant use. The seedlings are usually placed in small drainable plastic pots with peat and placed in the gutter (Figure 2). A thin film of 1-2 cm of nutrient solution flows along the bottom of the gutter (Somerville et al., 2014). The nutrients are mixed according to the plant's nutrient requirement in a reservoir from where it flows through the gutters to feed the plants preferably in a tilted position to facilitate nutrient solution flow in the gutters (Ezziddine et al., 2021). A basic requirement for lettuce growth in NFT is to provide all the nutrients the plant needs. According to Ezziddine et al. (2019), when lettuce is grown for more than six weeks with this system, there is depletion of macronutrients like N, P, K, and accumulation of slow absorbing nutrients like Ca, S, Zn, Cu, and B affecting yield, therefore, electrical conductivity (EC) and pH must be monitored and adjusted to required values to ensure proper growth and yield of plants.

The absorption of nutrients corresponds to the concentration of nutrients in the solution near the roots. The absorption of nutrients by the plants is influenced by factors like temperature, salinity, pH, light intensity, photoperiod, and air humidity. Excess or deficiency of dissolved elements in the nutrient solution will lead to deficiency symptoms or toxicity respectively (Domingues et al., 2012). According to R. Goto et al. (2001), lettuce absorbs small amounts of nutrients in their initial stage of growth compared to other cultures and increases demand for nutrients in the final stage of their life cycle. Supplying the required amount of nutrients at the right developmental stage is important in getting quality and improved yield. Therefore, this system requires in-depth knowledge of the plants' physiology and the factors that affect their growth (Savvas & Passam, 2002).

In greenhouse production, climate computer is used to control factors like temperature, air humidity, photoperiod, and so on.

*Figure 2: Example of newly transplanted lettuce seedlings in pots with peat growing in an NFT system in a growth chamber. Photo: Gifty Kodua*

## 2.4 Tipburn in lettuce

Tipburn is a major concern of lettuce growers. It is a physiological disorder seen as necrosis at the apex of leaf margins. According to Tibbitts et al. (1985), tipburn occurs because of weak cell walls or excessive turgor pressure within the laticifers causing the laticifer cells to burst. Localized calcium deficiency in these areas has been associated with the occurrence of tipburn since calcium is an important component of cell walls (Frantz et al., 2004). Tipburn occurs mainly in leafy vegetables like lettuce that are wholly or partly enclosed (Collier, 1982). It reduces the quality and shelf life of freshly harvested lettuce, thereby resulting in severe economic losses. Tipburn affects lettuce plants both in regulated environments and open fields. According to Barta & Tibbitts (1991a), tipburn is most severe and appears early in greenhouse and indoor production. However, cultivars differ in their susceptibility to tipburn because of differences in genetic makeup.

Calcium moves by transpiration mass flow in the xylem to tissues with high transpiration rates (Marschner, 2011). As plants transpire and take up water, nutrients (Ca, Mn, Mg, k) are taken up and used by the plant. Its deficiencies, therefore, cause cell death which is seen as necrosis on leaf margins (Tipburn).

There are two types of tipburn: inner leaf tipburn occurs when the tissue of young leaves' tip collapse and turns necrotic. Inner leaves have low transpiration rates and poorly developed

xylem to supply calcium to these areas (Barta & Tibbitts 1986). Since tipburn is associated with calcium deficiencies, suppressed transpiration resulting from factors like drought decreases calcium concentration in inner leaves leading to deficiencies. Also, the morphology of lettuce is such that, the inner leaves are more enclosed by outer leaves. This blocks air movement and creates humid conditions around the inner leaves. This humid environment leads to low transpiration and reduced calcium transport to the inner leaves. According to E. Goto & Takakura (1992), vertical air supply to the inner leaves can help with inner tipburn. Conversely, increase transpiration resulting in a higher growth rate leads to more outer leaf tipburn. According to Maruo & Johkan (2020), the transpiration rate of immature or inner leaves is less than mature or outer leaves, therefore the inner leaves of lettuce tend to be more susceptible to tipburn than the mature leaves because of deficiencies. However inner tipburn is more important to growers since it can be a gateway to bacteria and rejection of the whole product. On the other hand, plants with outer tipburn can still be sold when the outer leaves are removed but it is time-consuming.

When environmental requirements are at optimum, photosynthesis is maximized, which increases plant growth. Fast-growing leaves then get less calcium compared to what is needed for proper functioning. According to Maruo & Johkan (2020), this shortage is a result of an imbalance between the plant's calcium requirement for proper plant functioning and what is supplied by the roots during rapid development. Therefore, the condition can still occur even in calcium-rich media if plants experience water stress and low evapotranspiration.

Although calcium deficiency is the main factor causing tipburn in lettuce, it is influenced by environmental factors, such as light intensity, air temperature, and soil conditions. Since calcium moves by transpiration flow in the xylem, factors that cause low transpiration leads to a shortage of calcium in the fast-growing parts of the plant, especially factors that enhance rapid growth. This makes tipburn more of a stress-related disorder (Saure, 1998). Stressful conditions like unsuitable temperature, drastic changes in pH, low soil water content, and salinity can damage plant roots and prevent absorption of calcium. Furthermore, excess of other nutrients like $NH_4$ and K depresses the absorption of calcium causing deficiencies (Bierman et al., 1990).

Tipburn is assumed to occur if stress exceeds the plant's stress tolerance. However, stress below a damaging level (mild stress) may help increase plants' tolerance to tipburn and help reduce tipburn incidence (Saure, 1998).

Increased root pressure and volume flow in the xylem are necessary for transporting calcium into low transpiring areas like young leaves (Marschner, 2011). Low relative humidity causes stomata to close and prevent the movement of calcium to the shoots. Drought also causes plants to close their stomata and reduce water loss through transpiration. (Taiz et al., 2015).

To increase the calcium content of growing leaves, it is important to increase the transpiration rate of the plant rather than increasing the calcium content in the nutrient solution (Marschner, 2011). However, a high light sum can increase tipburn even if transpiration is increased. Increased incidence of tipburn (both outer and inner) has also been found when the light intensity is high.

High relative humidity (RH) and dark periods inhibit transpiration and favor directing the xylem flow to low-transpiring organs thereby decreasing tipburn in young leaves. This was also seen in the work of (Vanhassel et al., 2014), where night air humidity above 95% reduced tipburn in butterhead lettuce by 50% compared to 3% decreased when night air humidity was 65%. 24-hour airflow above 0.28 m·s-1 along cultivation beds was also found to be more effective than controlling air temperature in reducing tipburn in a closed plant factory (Lee et al., 2013).

## 2.5 Abiotic stress

Plants' environment presents them with many abiotic factors such as light, water, carbon dioxide, mineral nutrients, oxygen, humidity, temperature, and toxins during their growth and development. Fluctuations of these factors outside their normal can prevent the plant from achieving its full genetic potential. Plants' response to abiotic stress such as drought, heavy metals, extreme temperatures, salinity, and light fluctuations can be acute, which results in cell death and sub-acute, where there is induction of adaptive changes in biochemical and gene expression (Toivonen & Hodges, 2011). Since plants cannot move to escape an unfavorable environment, they alter their physical and developmental processes to maintain growth and reproduce when they find themselves in a stressful situation (Taiz et al., 2015).

According to Cramer et al. (2011), abiotic stress is a condition whereby plants' growth and yield is below optimum levels because of environmental conditions. Usually, plants are presented with different abiotic stresses together in the field. Different abiotic stress can lead to common cellular disorders and secondary stresses like membrane injury, reactive species (RS) damage, protein denaturation, and osmotic stress (primarily dehydration).

The response of plants to stress is stimulated by upstream signaling molecules like stress hormones [e.g., abscisic acid (ABA)], reactive oxygen species (ROS), polyamines (PAs), hydrogen sulfide ($H_2S$), nitric oxide (NO), phytochromes, and calcium ($Ca^{2+}$). These signaling molecules then mobilize downstream effectors, mainly protein kinases like mitogen-activated protein kinase (MAPK) and transcription factors like dehydration responsive element binding factor (DREB) to alter gene expression and enzyme activities (He et al., 2018) (Figure 3).

In response, plants use defenses like the cuticle (which is the outermost shield of plants), unsaturated fatty acids (UFAs) as membrane modulator and oxylipin precursor, reactive specie (RS) scavengers (which regulates RS homeostasis), molecular chaperones (which stabilize proteins and subcellular structures like a membrane) and compatible solutes (serving as osmoprotectants) inside cells (He et al., 2018) (Figure 3). In a situation where the plants are confronted with multiple stress, there can be interactions or crosstalk between hormones, secondary messengers, and protein kinases or phosphatase involve in each of the stress pathways. Therefore, the production of signaling intermediates in one stress response can affect the other (Taiz et al., 2015).

Response to abiotic stress can be dynamic and complex depending on the tissue or organ affected. It can also be reversible or irreversible. Long-term responses lead to adaptation while short-term responses lead to acclimation. In response to abiotic stress, plants will quickly down-regulate energy metabolism and protein synthesis. This helps them to conserve energy by shifting from growth to protective mechanisms to survive.

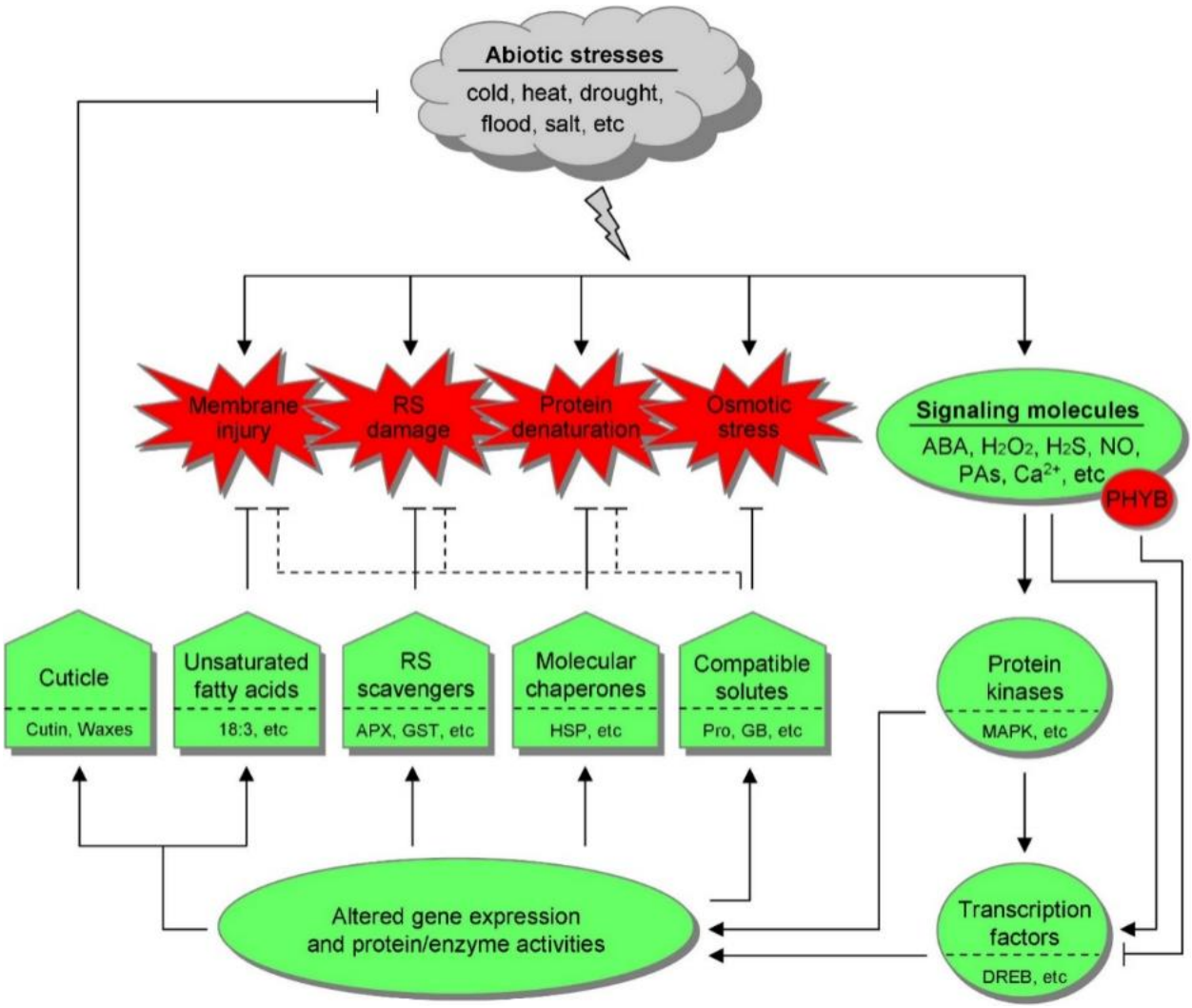



*Figure 3: General defense systems and the underlying regulatory network in plant's response to abiotic stresses. Some compatible solutes may also be involved in counteracting other adverse effects (dotted lines). Figure and test are taken from* (He *et al.*, 2018)

## 2.6 Reactive oxygen species (ROS)

Maintaining redox conditions is very important for physiology and development in aerobic organisms. During photosynthesis and via photosystem II (PS II), plants get electrons from water and liberate oxygen as a by-product. This process creates highly oxidizing species from which reactive oxygen species (ROS) can be formed in plant cells. ROS are also common by-products

of aerobic metabolism (Karuppanapandian et al., 2011). It is an important part of the regulatory networks that supports plant development and responses to the environment as they are excellent signaling molecules (Noctor et al., 2018).

ROS are highly reactive chemicals formed from molecular oxygen ($O_2$) when plants undergo oxidative stress. They are found in low and stationary levels in normal cells. ROS production is strongly influenced by stress factors like drought, salinity, chilling, defense of pathogens, nutrient deficiency, metal toxicity, and UV-B radiation in plants.

In vegetables, they are a part of the photoprotection process and increase plants' tolerance to stress. Although chloroplast is the main site of ROS production in photosynthetic cells (Asada, 2006) it can also be produced in mitochondria during the respiratory processes (Sies et al., 2017) (Figure 4). The production of ROS is kept in balance by various antioxidant systems (Karuppanapandian et al., 2011). For redox homeostasis, it is important to keep a balance between ROS and antioxidants for optimal plant growth. However, oxidative stress can shift the balance by increasing ROS and decreasing antioxidants which can result in enhanced oxidation resulting in cell death. It can also result in acclimation and improve plants stress tolerance, depending on the intensity of oxidative stress (Noctor et al., 2018). Too much ROS can reduce the endogenous antioxidant system resulting in oxidative stress and the formation of free radicals. The required level of ROS in cells is kept by the balance between ROS generating reactions and ROS scavenging reactions (Noctor et al., 2018).

While ROS generating occurs in cell compartments by specialized oxidases like NADPH oxidases, amine oxidases, and cell wall-bound peroxidases, ROS scavenging is carried out by antioxidant molecules such as ascorbate, glutathione, vitamin E, and carotenoids and by antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase and catalase (Taiz et al., 2015).

Since ROS such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($HO\cdot$) and singlet oxygen ($^1O_2$) are highly reactive, in the absence of any protective mechanism, ROS can damage cellular and molecular machinery, modify protein and lipid peroxidation, thus disrupting normal metabolism of plants leading to severe yield losses (Sies et al., 2017).

To avoid oxidative stress, it requires in depth knowledge of individual plants environmental requirements and physiology, to provide a stress-free environment for maximum yield.

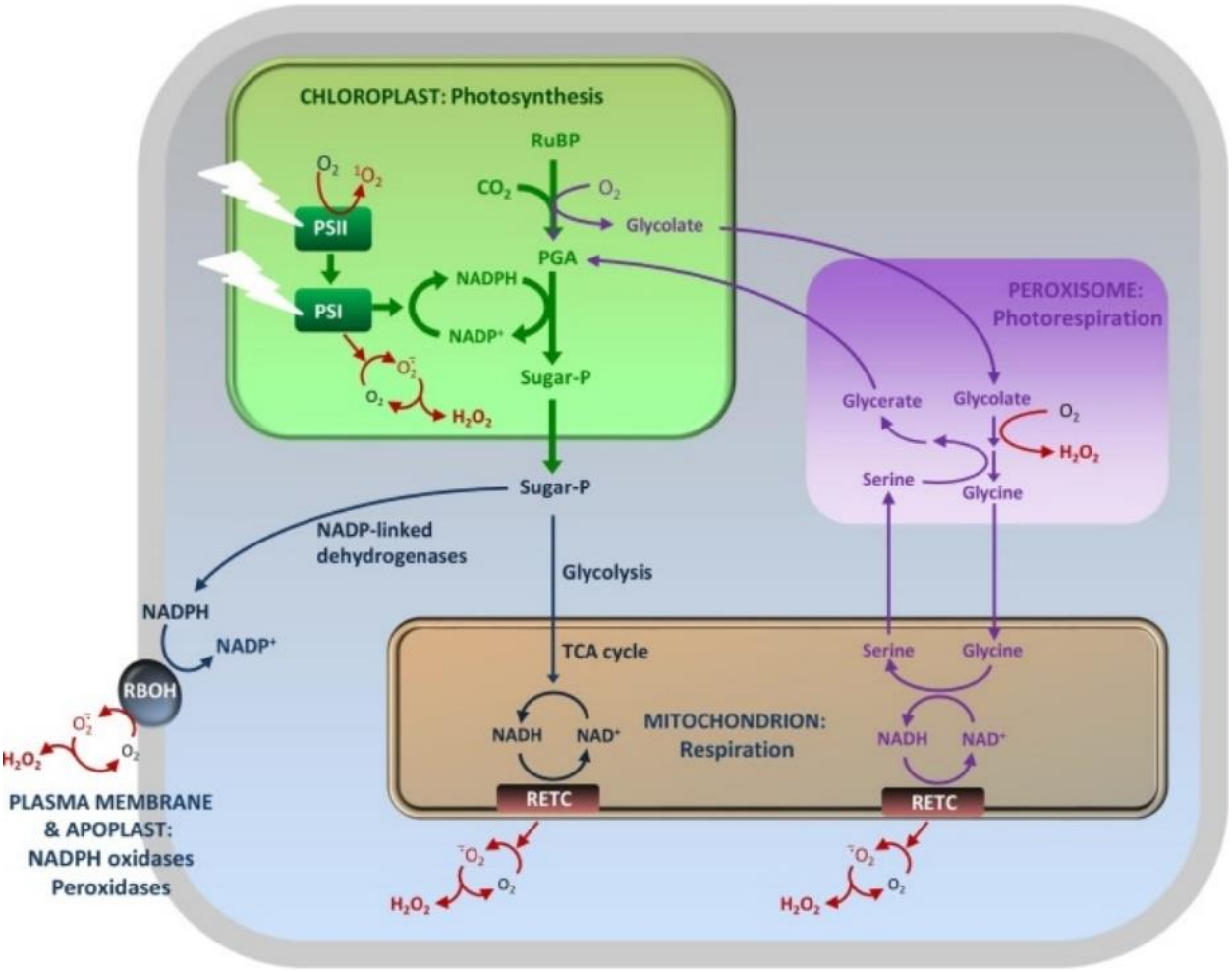



*Figure. 4: The basics of ROS formation in plants. The chloroplast is the main site of singlet oxygen formation whereas ROS generation by reduction of molecular oxygen occurs at several subcellular and extracellular sites. The figure and text were taken from (*Noctor *et al.*, 2018*).*

## 2.7 Antioxidants

In environmental stress like excess light, reactive oxygen species (ROS) accumulate in plant cells. These ROS are detoxified by specialized enzymes and antioxidants in a process called ROS scavenging. The detoxification enzymes and antioxidants function in cells as a network. This network is supported by several antioxidant recycling systems to refill the level of antioxidants to help maintain a safe level of ROS in cells and at the same time use it for signal transduction reactions (Taiz et al., 2015). Antioxidants and antioxidant enzymes protect plants against abiotic stresses (Foyer & Noctor, 2009).

According to Taiz et al. (2015), biological antioxidants are small organic compounds or small peptides that can accept electrons from ROS and neutralize them. Plant cells depend on reductases like glutathione reductase, dehydroascorbate reductase, and monodehydroascorbate reductase which use the reducing power of NADH/NADPH produced by respiration or photosynthesis to maintain antioxidants like water-soluble ascorbate (vitamin C), reduced tripeptide glutathione (GSH in reduced form, GSSG in oxidized form), lip soluble αtocopherol (vitamin E) and β-carotene (vitamin A).

Plants also produce antioxidative enzymes like superoxide dismutase (SOD), POD, catalase (CAT), and ascorbate peroxidase (APX) in response to ROS production to avoid oxidative stress and cell damage. Also, plants produce these antioxidants to maintain a balance between ROS production and its removal for optimum photosynthesis (Foyer & Noctor, 2009).

According to Kreslavskii et al. (2016), red light increased antioxidants like carotenoids and UV-absorbing pigments in spinach. Therefore, choosing the right wavelengths/light quality is important to optimize crop production (Shafiq et al., 2021).

## 2.8 Manganese in plants

Manganese is an essential micronutrient that was first discovered in the ash of vegetables. It exists in biological systems as Mn II, III, and IV with Mn (II) and Mn (IV) being stable and Mn

(III) unstable (Hughes & Williams, 1988). However, plants can take the divalent form ($Mn^{2+}$) as Mn (III) is unstable and Mn (IV) forms highly insoluble oxides and precipitates (Schmidt & Husted, 2019).

Manganese is a fast ligand exchanger that can easily replace other divalent metal ions, like Mg, Ca, Fe, Co, Cu, and Zn. Therefore, excessive concentrations of Mn in plant tissues may change various processes, like enzymatic activity, uptake, redistribution, and the use of other nutrients like Ca, Fe, Mg, N, and P in addition to the productive responses of agricultural crops (Lavres Junior et al., 2010).

Mn transport occurs mainly in the Xylem transport from roots to the above-ground parts of plants by the transpiration stream accumulating mainly in the shoots. It is less mobile in the phloem and takes place from sources to sinks (Marschner, 2011). Redistribution depends on the plant species and stage of development (Millaleo et al., 2010).

Mn bioavailability in the soil is influenced by pH and redox conditions, where its concentration increases with low pH (< 5.5) and increased redox potential, and reduces with increased pH (pH 8) (Millaleo et al., 2010). Plants species and parts differ in their quantity of manganese (McHargue, 1922). Depending on Mn availability and transport processes, plants either efficiently use limited supply or detoxify superfluous supply.

### 2.8.1 Manganese (Mn) deficiency and toxicity in plants

In plants, Mn is involved in processes like photosynthesis, respiration, scavenging of reactive oxygen species (ROS), pathogen defense, and hormone signaling. it is also a cofactor for many metalloenzymes (Waldron et al., 2009) activating those enzymes in plants. In these enzymes, Mn has two major functions; as a Lewis acid (it can accept a pair of electrons (a lone pair) from a donor molecule and form a coordinate covalent bond) and an oxidation catalyst (Schmidt & Husted, 2019).

Mn is part of the enzymes such as Mn superoxide dismutase (Mn-SOD), oxalate oxidase ($O_xO_x$), and the Mn protein in photosystem II (PS II) (Schmidt & Husted, 2019). That is, these enzymes need Mn to become catalytically active (Zhu & Richards, 2017). Mn protein in photosystem II is

an important metal cofactor in the oxygen-evolving complex (OEC) of higher plants which forms part of photosynthetic protein and enzymes that catalyzes the oxygen-evolving complex in photosystem ll (Millaleo et al., 2010). During photosynthesis, tetra-Mn cluster $Mn_4O_5Ca$ splits two molecules of water into four electrons, four protons, and molecular $O_2$ (Bricker et al., 2012) which provides electrons for driving photosynthesis. Mn deficiency, therefore, affects photosynthesis.

Superoxide dismutase (SOD) is an essential enzyme in the survival of aerobic organisms when oxygen is present. The superoxide dismutase (SOD) enzyme is involved in scavenging or detoxifying reactive oxygen species (ROS) in plants (Schmidt & Husted, 2019). It catalyzes the dismutation of superoxide radicals into molecular oxygen ($O_2$) and hydrogen peroxide ($H_2O_2$). This protects the plant tissues from the negative effect of oxygen free radicals $O^{-}{}_{2}^{\cdot}$ formed in reactions where a single electron is transmitted to $O_2^{\cdot}$.

Plant SOD may use Cu (Cu-Zn-SOD), Fe (Fe-SOD), or Mn (Mn-SOD). Among these, Mn-SOD has a greater reduction potential. Changes in SOD activity indicate the level of ROS production and oxidative stress (Foyer & Noctor, 2009). Mn-SOD is mainly found in mitochondria and peroxisomes of plants (Marschner, 2011). During superoxide oxidation, an electron is transferred to $Mn^{3+}$ to produce $Mn^{2+}$-SOD and $O_2$ (Zhu & Richards, 2017). In an experiment by (Leonowicz et al., 2018), the Mn-SOD activity was high in the tip of wheat leaves. This indicates the high production of ROS production in peroxisomes or mitochondria in aging mesophyll cells. Mn deficiency in green algae Chlamydomonas resulted in no Mn-SOD activity prior to PSII reducing its efficiency (Allen et al., 2007). That is, prolonged Mn deficiency seen as necrosis on leaves may be because of decreased levels of Mn-SOD and increased free oxygen radicals. $Mn^{2+}$ cation itself may act as an antioxidant molecule (Alejandro et al., 2020).

Oxalate oxidase (Mn containing enzyme) is deployed by plants in defense against pathogen attack by $H_2O_2$-mediated lignification. This enzyme is used in various breeding programs to increase plants resistance to pathogen attack and thereby reduce diseases in crop production.

Mn concentration below 10–20 mg. $kg^{-1}$ dry weight leads to deficiency in the plants (Broadley et al., 2012). This affects growth and decreases plant biomass. Deficiencies also lead to higher

susceptibility to pathogen infections, reduced number of Mn-complexes in the PSII core, imbalance in plant water relations, and decreased tolerance to low temperatures. Mn deficiency appears first in younger leaves because of low phloem mobility. Deficiency is characterized by pale mottled leaves and interveinal chlorosis which turn brownish or necrotic. Roots develop more root hairs and become necrotic under serious deficiencies (Yamaji et al., 2013)

Since Mn availability is affected by pH, alleviating Mn deficiencies at the soil level is effective if soil pH is corrected (White & Greenwood, 2013). Foliar Mn application can also be an effective way to control deficiencies, but it is limited in its efficiency since Mn is less mobile in plants and does not remobilize from older leaves to Mn-deficient young leaves (Li et al., 2017).

Mn toxicity is seen as chlorosis, small dark spots, necrosis, leaf distortion, and abscission on leaves. These symptoms vary among plant species. The difference in Mn tolerance and hence the expression of toxicity symptoms among plant species is due to differences in Mn uptake and translocation which also depends on the plant's genotype, coupled with developmental and environmental factors in which the plant is growing (Horst, 1988). Toxicity causes the degradation of lipids, proteins, carbohydrates, and nucleic acids. This leads to damaged cell metabolism and in some cases causes cell death (Fernando et al., 2015).

In leaf tissues, the toxic effect of excess Mn is because of Phyto oxidative stress, callose formation, and disruption of electron flow in the chloroplast (Fernando & Lynch, 2015). This may alter processes like enzymatic activity, uptake, redistribution, and use of other nutrients. Toxicity occurs in poorly drained and strongly acidic soils where it is usually associated with other acidity-related soil fertility problems, such as aluminum toxicity and deficiencies of calcium, magnesium, and molybdenum (Goulding, 2016).

## 2.9 Light – intensity, photoperiod, and spectral distribution

Light is the main source of energy for photosynthesis and many other physiological processes affecting plant growth. Radiation reaching the earth's surface ranges between wavelengths of approximately 300 – 2500 nm. For crops, wavelength range from 400 – 700 nm known as

photosynthesis active radiation (PAR) is the part plants use for photosynthesis (Ahmed et al., 2020). PAR is determined by the absorption spectra of photosynthetic pigments involved in capturing light. Other wavelengths like ultraviolet, 300 – 400 nm, and near-infrared, 700 – 2500 nm are used by plants in other developmental processes. The radiation consists of particles called photons. The energy of a photon is inversely proportional to its wavelength. However, photons of wavelength in the PAR range contribute equally to photosynthesis (Stanghellini et al., 2019). Maximum irradiances receive by plants at a given time vary because of differences in latitudes and daily and annual cycles (Willey, 2015).

Greenhouses use the sun as a free light energy source, but the amount of daylight received from the sun in the greenhouse is reduced by greenhouse coverings. Light intensity, spectra, and distribution affect plants' growth and development. In the greenhouse, light is used in photosynthesis and heating. Too much or too little light can stress greenhouse vegetables that have specific light requirements (Gruda, 2005)

During photosynthesis and transpiration, light intensity affects the transport of $CO_2$ and $H_2O$ through the stomata (Shibata et al., 1994). Optimum light, therefore, increases the photosynthetic rate and dry mass production (Bian et al., 2015). During winter, Photosynthetic Photon Flux Density (PPFD) level is reduced to a level that limits photosynthesis and growth. Supplementary light is, therefore, necessary for greenhouse production, especially in Norway where there are long periods when the natural solar radiation is too low for plant production. Supplementary light increases nutrient and water uptake compared to natural light (Dorais, 2003).

According to Ahmed et al. (2020), light spectral distribution is the portion of light with red, blue, green, or other visible or invisible wavelengths. Specific plant functions occur at different wavelengths. Plants have phytochromes and absorbing pigments to sense and respond to light spectra. Chl a and b are the primary photosynthetic pigments in plants. They have their peak absorption in red and blue wavelengths. Therefore, red (610-760 nm) and blue (450-500 nm) lights particularly affect the photosynthetic rate and growth of crops. Blue light is important in stomatal opening. Far-red light does not contribute very much to photosynthesis due to low absorption.

Although far-red light is less absorbed by plant pigments, it affects the germination and flowering of plants. Also, Far-red light and green light induce leaf enlargement, leaf elongation, and leaf upward orientation altering the plant form (Zhang et al., 2011).

The duration of light (photoperiod) affects plants in every stage of their growth from germination to flowering. Proper control of the lighting environment (intensity, spectral distribution, and photoperiod) for lettuce production in a controlled environment with artificial lighting is an effective technique to increase growth rate and quality and achieve the highest light use efficiency.

### 2.9.1 Lamp types

#### 2.9.1.1 High pressure sodium (HPS)

In high latitudes like Norway, high pressure sodium (HPS) lamps are the main source of assimilation lighting in greenhouses (Marcelis et al., 2019). HPS is a high intensity lamp. It has a maximum efficiency of about 1.85µmol J-1 (Stanghellini et al., 2019). 400W, 600W 1000W lamps are used. A 1000W HPS lamp (the most efficient and preferred by farmers) converts about 37% of its electrical energy provided into PAR, 39% into heat, 5% in electrode and 18% is mainly thermal infrared. 20–22% of its electrical input is converted into near infrared. The thermal energy produced reduces the demand from the greenhouse heating system and helps increase plant temperature (Gomez et al., 2013).

HPS offers a broad spectrum of light and can therefore be used in all kinds of crops. It has a large component of yellow-red light. When using HPS, it is important to keep some distance between the lamp and the plants to avoid tissue scorching from the thermal energy. To get well distributed and most of the light directed towards the crops, the lamp must be fitted into a reflector.

#### 2.9.1.2 Light emitting diodes (LEDs)

LEDs provide a specific light spectrum and come in a variety of wavelengths. This makes it possible to select wavelengths for specific morphological or physiological plant responses. There is red/blue and white spectrum light. Red and blue light is most important for photosynthesis and plant growth. It produces as much as 3 μmol $J^{-1}$ (Stanghellini et al., 2019) and converts 30%–70% of its electrical energy into PAR, and 0–2% into near infrared (NIR) (Katzin et al., 2020). Its efficacy mainly depends on the spectral output of the lamp.

LEDs operate at a considerably lower temperature resulting in low far infrared radiation (FIR), (Katzin et al., 2020). The lower far infrared radiation (FIR) makes it possible to place LEDs close to plants to increase available PAR at leaf level without overheating or scorching plant tissue because of the coolness of its surface (Gomez et al., 2013) but you must put in more heating system to increase the temperature in the greenhouse. Also, lack of thermal radiation causes lower crop temperature thereby lowering productivity. This can be corrected by raising the air temperature in the greenhouse.

### 2.9.2 Light stress and excess manganese (Mn)

Plants use light energy for photosynthesis and as a signal for developmental processes like germination, shade avoidance, stomatal development, circadian rhythm, and flowering (photomorphogenesis) (Müller-Xing et al., 2014). The light intensity can be as high as ˜2000 μmol photons $m^{-2}$ $s^{-1}$ in full sunlight and negligible in a deeply shaded environment (Yang et al., 2019). Fluctuations or deviation from optimum light requirement (both intensity and spectral quality) of plants causes stress and affects photosynthesis negatively thereby affecting the plant's growth and yield (Nishiyama & Murata, 2014). If plants are exposed to excess light, they experience photoinhibition (Willey, 2016).

The reaction center of photosystem ll (PS II) has been found to be more sensitive to photoinhibition. Excessive light also causes the production of reactive oxygen species produced in the chloroplasts, which inactivates the photochemical reaction center of PSll leading to

photodamage. The photodamage to PSII is linked with light absorbed by the manganese cluster of the oxygen-evolving complexes (Zavafer et al., 2015). In high lights, the chloroplast is mostly affected.

Response to stress depends on light frequency, photoperiod, and priming. However, it is affected by factors like plant leaf age, temperature, light intensity, soil nutrient balance, soil pH, and genotype. In response to long term fluctuating light intensities, plants may orient their leaves differently, change their stomatal counts and densities or alter enzyme activities to adapt their growth and metabolism in an optimal way to unpredictable light conditions (Yang et al., 2019). Chloroplast movement has been found in response to high or low light situations at the cell level, where the chloroplast moves to the side of the cell to reduce the amount of light it absorbs when light intensity is high (Kasahara et al., 2002) and distribute chloroplasts in the cell to maximize the light capturing when light intensity is low (Briggs & Christie, 2002).

Excess of Mn (Mn toxicity) induces oxidative stress causing symptoms like chlorosis, callose formation, and necrosis. The effect of light intensity on Mn-toxicity symptoms differs among crops. Most crops have shown fewer symptoms of Mn toxicity in low light than in high light (González et al., 1998). Induced chlorosis by excess Mn is attributed to photooxidation of chlorophyll (Gerretsen, 1950) suggesting less photo destruction of chlorophyll in plants grown in low light. Also, fewer toxicity symptoms seen in plants grown in low light is because plants that are grown in low light usually accumulate less foliar Mn than those grown at a higher light intensity (González et al., 1998). Light intensity also affects antioxidants, antioxidant enzymes, and chlorophyll content. In the work of González et al. (1998), common beans grown with excess Mn in high light have increased activity of antioxidant enzymes, ascorbate peroxidase, and superoxide dismutase. Cucumis sativus L. treated with excess Mn under optimum light intensity showed increased activity of superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and glutathione reductase, and dehydroascorbate reductase (Shi et al., 2006). Increased Mn with increased light may intensify oxidative stress and cause growth inhibition.

# 3 Materials and methods

## 3.1 Planting material and pre cultivation

Coated seeds of Frillice' lettuce (Lactuca sativa L. `Frillice´) from Norgro (Norway) were used in this experiment. The seeds were sown to about half centimeter depth in small drainable biodegradable plastic pots of about 0.08litre capacity filled with fertilized peat (" Degernes torv" type of peat from Degernes Torvstrofabbrikk AS, Norway).. The sown seeds were watered with pure water and covered with an aerated net to ensure enough moisture and create darker environment for better germination of the seeds (Figure 5). They were kept in a dark room with a temperature of $15^0$C and relative air humidity (RH) of 60% for four days for germination.


***Figure 5:*** *Sown seeds covered with a net in the dark room for germination (top); and tray with drainable biodegradable plastic pots containing peat with the germinated seed at the greenhouse at day 5 where plants will be grown to reach five leaf stage before they are moved to the growth chamber for their final days in the experiment (bottom left). A bag of peat type that was used in all the experiments (bottom right). Photo: Gifty Kodua.*

After germination, the seedlings (Figure 5) were moved to the greenhouse (controlled environment) for further growth. The plants grew in the green for about three weeks until they have reach 5 leaf stadia (5 true leaves) before they are moved to the growth chambers. In the greenhouse, the seedlings were given 18 hours of lighting from 400 watts HPS (High Pressure Sodium lamps, from Gavita, Norway) with a photon flux density (PFD) of 150 µmol $m^{-2}$ $s^{-1}$. The temperature was $20^0$C with a relative air humidity of 60%. The temperature and air humidity were maintained throughout the day and night. The seedlings were watered at least once a day with a greenhouse fertilizer solution with electrical conductivity (EC) of 1.5 mS $cm^{-1}$ using a watering can.

Priva climate computer (Priva, Zijlweg, The Netherlands) was used to control the climate in the greenhouse. Water from sprinklers in the roof was used to humidify the air when necessary. 300 Watts irradiance was the threshold for turning the sprinklers with effervescence lasting for 10 seconds.

## 3.2 Growth chamber set-up/Getting growth chamber ready

A closed growth chamber with a controlled environment was used in all the experiments. In all the chambers, a hydroponic system-nutrient film technique (NFT) was installed. The source of light used in the growth chambers was high pressure sodium (HPS) or light emitting diodes (LED) lamps (Evolys, Norway). The HPS lamps provided 400 Watts (provided by Gavita, Norway).

The chambers had four metal gutters (Vefi AS, Norway) resting on a metal stand. The gutters were 1.5 m long and 10 cm wide. The metal gutters were slightly tilted using Draper 300mm box section spirit level to ensure that excess water is drained into a plastic container beneath the stand (Figure 6). The gutters had 10 holes each for the transplanted plants to fit in. The holes were about 15cm in diameter and 25 centimeters apart for proper aeration and optimum light. One end of the gutter was connected to an adjustable pump (Aquarium Systems Maxi-Jet 500, France) with a timer (müeller SC 28 11 pro, Germany) which sucks up a nutrient solution from a black plastic box placed under the metal stand (Figure 6). Every minute pump provided about 130ml of the nutrient solution in each gutter.

The slightly tilted metal gutters were open at the end to let out the excess nutrient solution. Two transparent plastics were placed on the floor in alignment with the metal gutters to collect the excess water (Figure 6). A climate sensor box connecting to a climate computer to control and measure air humidity and the temperature was hanged at the ceiling in the chamber (The sensor box had both dry and wet sensors).

***Figure 6:*** *Showing the spirit level (left) used to get a slight tilt of the metal gutters (left), aligning the plastic container that collects excess nutrient solution (middle) and black plastic box with adjustable pump for nutrient solution, hanging from the top is the timer (right). Photo: Gifty Kodua*

***Figure 7****. Ready seedlings from the green house ready to be distributed in the gutters (top left and bottom), distributed seedlings in the gutters at the chamber [(top right) (hanging from above is the water timer)]. Photo: Gifty Kodua*

## 3.3 Lighting

Growth chambers had either light emitting diodes (LED) or high pressure sodium (HPS) lamps at the ceiling (top lighting) according to the aim of the experiment. HPS lamps used were 400 watts each from Gavita, Norway (Figure 8).

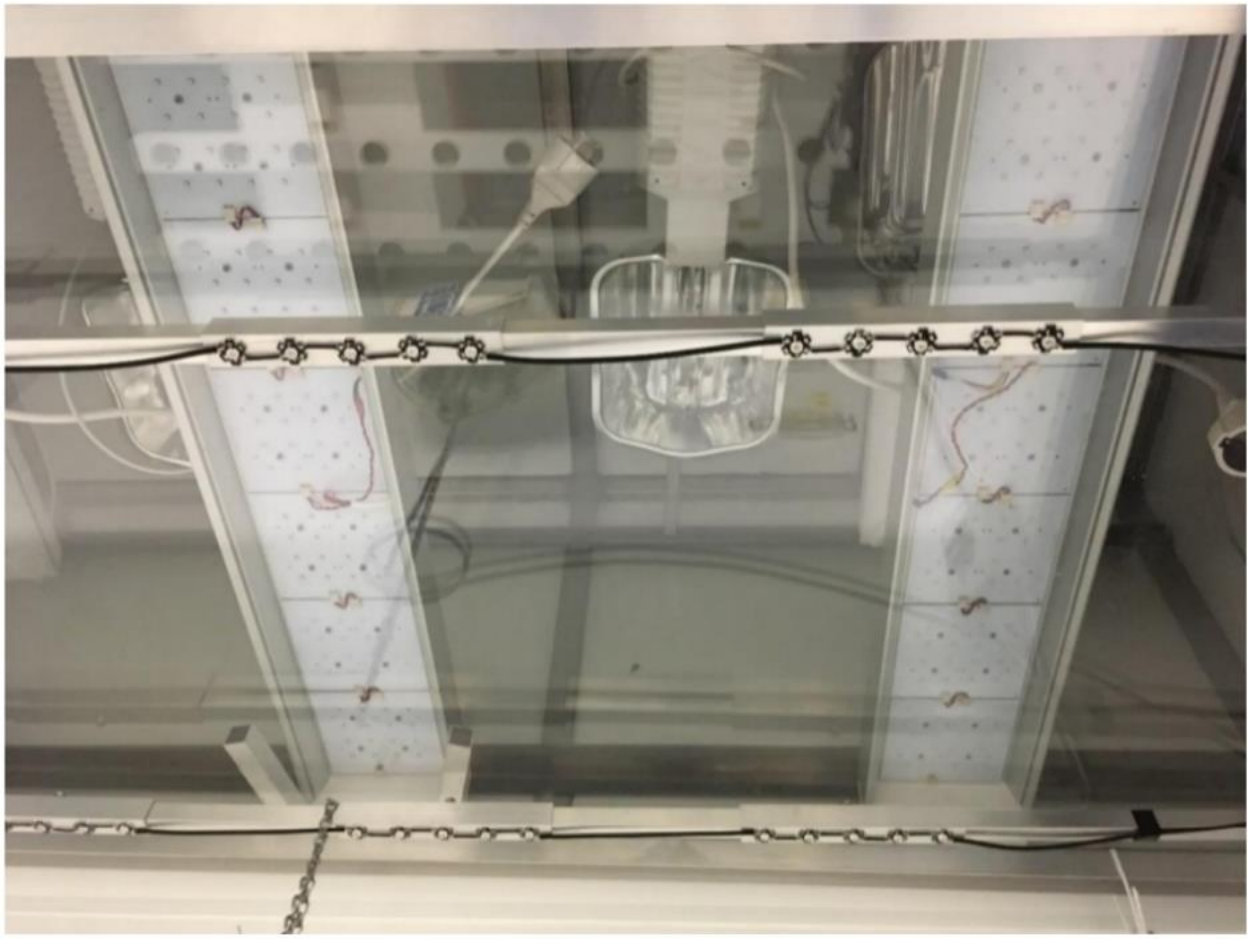
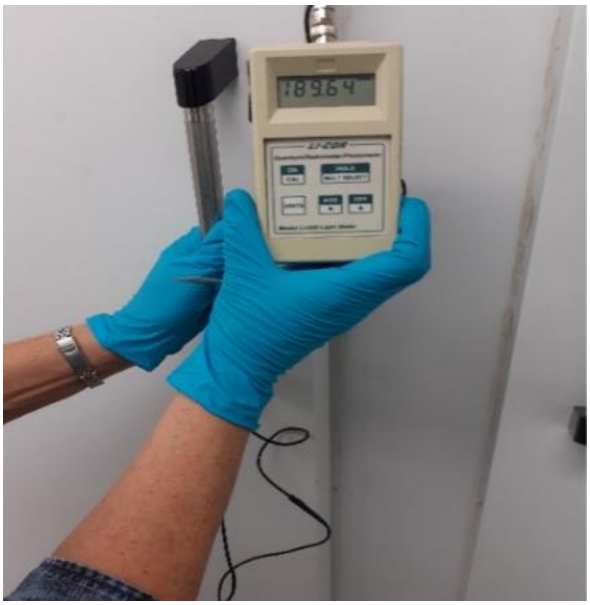


***Figure 8:*** *Roof of a chamber with two HPS lamps (400 watts each, vertical) and LED (185 watts each, vertical) with two dimmable far-red (80 watts each, horizontal). Photo: Martin Knoop*

### 3.3.1 Photosynthetic Photon Flux Density (PPFD)

The amount of PAR light (photons) emitted by the LED and HPS in the chambers was measured with a quantum meter (Li-250A light meter, Li-Cor, USA), for 400-700 nm wavelengths. Measurements were taken with the chamber doors closed (Figure 9a).



***9a*** ***9b.***

***Figure 5a:*** *Optronic model 756 spectroradiometer use in measuring spectral compositions Photo: Martin Knoop.*
***Figure 5b:*** *A quantum meter (Li-250A light meter, Li-Cor, USA) use to measure light intensity/photon flux in the chamber. Photo: Gifty Kodua*

In chambers with HPS lamps, a net (net added to reduce irradiance and net removed to increase irradiance) was used at the ceiling to adjust the irradiance from the lamp to get the correct flux with the quantum meter whilst chambers with LED were adjusted manually with regulators on its monitor. Measurements were taken at different places in the chamber which varied slightly but kept within +/- 10% of the stated mean.

### 3.3.2 Spectral distribution/composition

Optronic model 756 spectroradiometer (Optronic Laboratories, Orlando, FL, USA), (Figure 9b) was used to measure spectral compositions and irradiance levels of the optical radiation sources (UV-visible-infrared) for the HPS and LED as explained in (Suthaparan et al., 2018) method 46.

### 3.3.3 Red/far-red ratio (R/FR-ratio)

R/FR-ratio was measured with a red/far-red sensor (Skye red/far-red sensor, The UK), at 660nm and 730 nm wavelengths. The measurement was done the same way the photon flux was measured with a quantum meter. R/FR-ratio for HPS is stated to be 3.7 (Gavita, Norway).

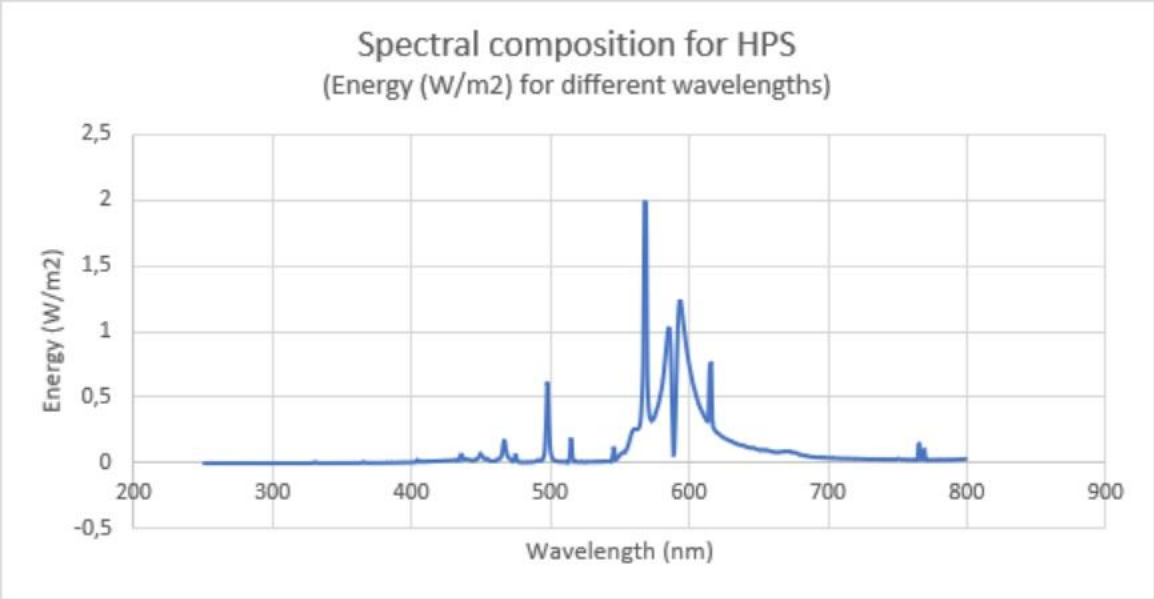



*Figure 10: Spectral composition for 400 W HPS, (Gavita Norway) used in growth chambers for experiments 1 and 2. Credit: Martin Knoop.*

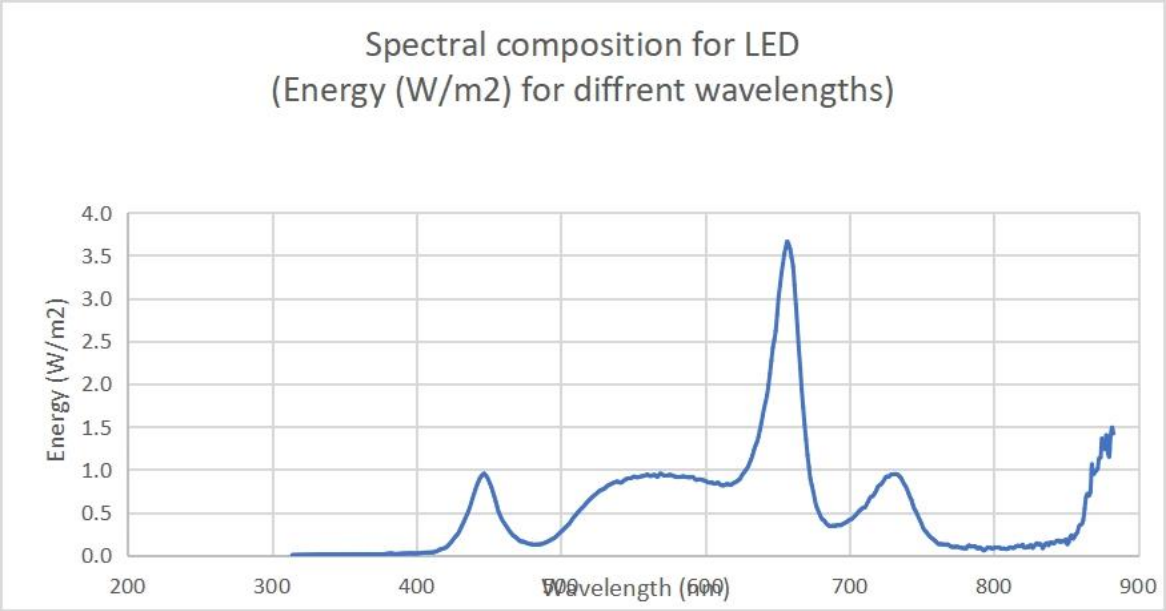



*Figure 11: Spectral composition for LED used in growth chambers for experiments 3, 4, and 5.*

## 3.4 Nutrient solution for watering/fertilization

The nutrient solution that run through the gutters to fertilize the plants in the chambers was of two types. One was labeled normal nutrient solution without extra Mn (-Mn), and the other was labeled nutrient solution with extra Mn (+Mn). The normal nutrient solution was prepared from two different stock solutions (stock I and stock II) (Table 2) whilst the other solution had an extra 15.25 g of $MnSO_4$ (111ppm of Mn) added to the normal solution.

### 3.4.1 Preparation of nutrient solutions

50 litters of tap water kept overnight was fetched into a container. The two stocks (stock I and stock II) were added in equal proportions to the water whilst stirring until an electrical conductivity (EC) of 2.0 mS $cm^{-1}$ was attained. About 750-1000ml each of stocks 1 and 2 was added and thoroughly mixed with a wooden stick to attain the electrical conductivity of 2.0 mS $cm^{-1}$. An EC meter (ScanGrow Conductivity meter, Denmark) was used in measuring the EC of the solution (Figure 12). It was labeled a normal nutrient solution (0.5 ppm Mn). This was finally

fetched into the black container beneath the metal stands in the growth chamber and refilled when necessary.

The second nutrient solution was prepared the same way as described for the normal nutrient solution but had an additional 15.25g of $MnSO_4$ (111ppm Mn). This was thoroughly mixed with a wooden stick to obtain an EC of 2.2 mS $cm^{-1}$. This was to investigate the influence of extra Mn in nutrient solution on the occurrence of tipburn on lettuce growing in different light intensities and lamp types.

*Table 1: Composition of stock I and II used in preparing the nutrient solution*

| **Stock I** | | **Stock II** | |
|---|---|---|---|
| Type | Amount | Type | Amount |
| Calcium nitrate | 2.5 kg | Pioneer basic cucumber | 3.125 kg |
| Potassium nitrate | 0.625 kg | Pioneer Iron chelate, 6% EDDHA | 0.025 kg |
| Calcium chloride | 0.15 kg | | |

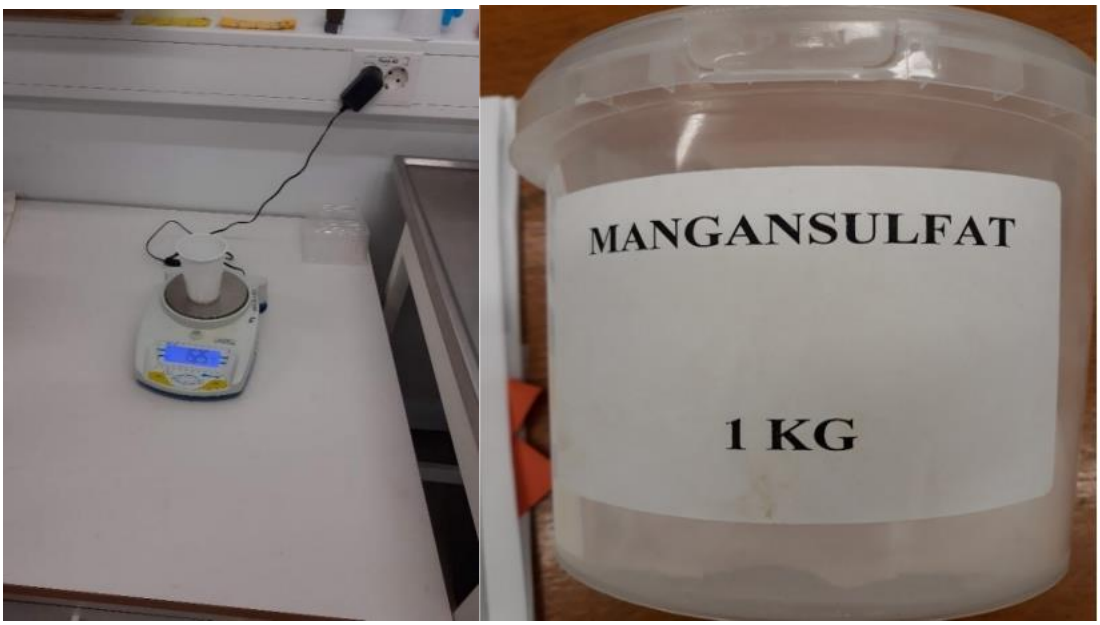




*Fig 12: Final nutrient solution with extra Mn with electrical conductivity of 2.2 mS $cm^{-1}$ (top left), an EC meter used in measuring the electoral conductivity of the final solution (top right), 111ppm of extra Mn that was added (bottom left), manganese used in the solution (bottom right). Photo: Gifty Kodua*

## 3.5 Experiment 1- Effects of extra Manganese (Mn) in the nutrient solution on tipburn and growth of lettuce grown in high and low irradiance provided by HPS lamps

Two treatments were under study. The experiment started with five leaf stage plants. The plants were pre-cultivated as described above. Each treatment had 24 plants. During the first two weeks of the experiment, plants were grown at moderate irradiance (150 µmol $m^{-2}$ $s^{-1}$) with 400watts HPS and watered with a normal nutrient solution for two weeks (Table 2). The nutrient solution was pumped for one minute (10 times during the photoperiod) at a 2-hour interval.

After two weeks, both treatments received nutrient solution with extra Mn (111ppm). The irradiance in one of the chambers was increased to 300 µmol $m^{-2}$ $s^{-1}$. At this time, the nutrient solution was pumped for two minutes, 10 times during the photoperiod in the treatment with increased irradiance. The treatment with moderate irradiance continued with the same watering program. Other factors were the same and remained constant in both treatments throughout the experiment (Table 3).

*Week 1 and 2*

*Table 2: Details of treatments in experiment 1.*

| Treatment | Lamp type | Temp, Day (˚C) | Temp, Night (˚C) | Relative Humidity (RH) | Photon flux density (µmol $m^{-2}$ $s^{-1}$) | Photoperiod (hr) | Extra Mn ppm (mg/l) |
|---|---|---|---|---|---|---|---|
| Chamber 1 | HPS | 20 | 18 | 70% | 150 | 18 | - |
| Chamber 2 | HPS | 20 | 18 | 70% | 150 | 18 | - |

*Table 3: Details of treatments used in experiment 1 in weeks 3-4. High light + extra Mn (HL/EMn), moderate light + extra Mn (LL/EMn).*

| Treatment | Lamp type | Temp, Day (˚C) | Temp, Night (˚C) | Relative Humidity (RH) | Photon flux density (µmol $m^{-2}$ $s^{-1}$) | Photoperiod (hr) | Extra Mn ppm (mg/l) |
|---|---|---|---|---|---|---|---|
| LL + EMn | HPS | 20 | 18 | 70% | 150 | 18 | 111 |
| HL + EMn | HPS | 20 | 18 | 70% | 300 | 18 | 111 |

The plants grew for two weeks under the different treatment before the experiment ended. A random sampling of 10 plants from each treatment was taken for tipburn assessment and growth rate (fresh weight, dry weight, leaf number, and length of longest leaf). Individual leaves were assessed for tip burn severity. For details of how growth and tipburn assessment was done, see chapter 3.10.1. Another five different plants were again selected at random from the two different treatments for nutrient analysis. See details on how samples were prepared for nutrient analysis in chapter 3.11.

## 3.6 Experiment 2- Effect of extra Manganese (Mn) in the nutrient solution on tipburn and growth of lettuce when moving plants from low to high irradiance using HPS lamps as a light source

In experiment 2, two treatments were under study. The plants were pre-cultivated as described above. 24 plants were used for each treatment. During the first two weeks of the experiment, plants were grown at moderate irradiance (150 µmol $m^{-2}$ $s^{-1}$) with 400watts HPS. Nutrient

solution with salinity 2.2 mS $cm^{-1}$ with extra Mn (111ppm) was applied in one chamber. The other chamber had only nutrient solution (0.5ppm Mn) with a salinity of 2.0 mS $cm^{-1}$ (Table 4). Water was pumped for one minute 10 times during the photoperiod.

After two weeks the light for both treatments was increased to 300µmol $m^{-2}$ $s^{-1}$ while the other factors remained the same (Table 5). At this time, the water was pumped for two minutes 10 times during the photoperiod. Growing conditions in both chambers were the same and remained constant throughout.

*Table 4: Details of treatments used in week 1-2 of experiment 3. Low light + extra Mn (LL/EMn), Low light without extra Mn (LL/wEMn).*

| Treatment | Lamp type | Temp, day (˚C) | Temp, night (˚C) | Relative Humidity (RH) | Photon flux density (µmol $m^{-2}$ $s^{-1}$) | Photoperiod (hr) | Extra Mn ppm (mg/l) |
|---|---|---|---|---|---|---|---|
| LL/EMn | HPS | 20 | 18 | 70% | 150 | 18 | 111 |
| LL/wEMn | HPS | 20 | 18 | 70% | 150 | 18 | - |

*Table 5: Details of treatments in week 3 and 4 of experiment 3. High light + extra Mn (HL/EMn), High light without extra Mn (HL/wEMn).*

| Treatment | Lamp type | Temp, day (˚C) | Temp, night (˚C) | Relative Humidity (RH) | Photon flux density (µmol $m^{-2}$ $s^{-1}$) | Photoperiod | Extra Mn ppm (mg/l) |
|---|---|---|---|---|---|---|---|
| HL/EMn | HPS | 20 | 18 | 70% | 300 | 18 | 111 |
| HL/wEMn | HPS | 20 | 18 | 70% | 300 | 18 | |

At the end of the experiment, 10 plants were sampled at random for each treatment for tipburn assessment and growth rate (fresh weight, dry weight, leaf number, and length of longest leaf). Individual leaves were assessed for tipburn. For details of how growth and tipburn assessment was done, see chapter 3.10.1. Another five different plants were again selected at random from the two different treatments for nutrient analysis. See details on how samples were prepared for nutrient analysis at chapter 3.11.

## 3.7 Experiment 3- Effect of extra Manganese (Mn) in the nutrient solution on growth and tipburn of lettuce growing in low irradiance using LED lamps as a light source

In experiment 3, the effect of extra Mn on growth and occurrence of tipburn under low irradiance with LED light was studied (Table 6). The plants were pre-cultivated as described above. 24 plants were used for each treatment. Plants in one chamber received the nutrient solution of salinity 2.2 mS $cm^{-1}$ with extra Mn (111ppm). The other chamber (treatment) had only nutrient solution (with 0.5 ppm of Mn) with a salinity of 2.0 mS $cm^{-1}$ without extra Mn (111 ppm). Water was pumped for one minute 9 times during the photoperiod for both treatments. Conditions in both chambers were the same and remained constant throughout the experiment (Table 6).

***Table 6:*** *Details of treatments used in experiment 3. Low light + extra Mn (LL/EMn), Low light without extra Mn (LL/wEMn)*

| Treatment | Lamp type | Temp, day (°C) | Temp, night (°C) | Relative Humidity (RH) | Photon flux density (µmol $m^{-2}$ $s^{-1}$) | Photoperiod (hr) | Extra Mn ppm (mg/l) |
|---|---|---|---|---|---|---|---|
| LL/EMn | LED | 20 | 18 | 70% | 134 | 16 | 111 |
| LL/wEMn | LED | 20 | 18 | 70% | 134 | 16 | - |

The plants grew in the chambers for four weeks and the experiment was terminated. 10 plants were randomly sampled from each treatment for tipburn and growth assessment. For details of how growth and tipburn assessment was done, see chapter 3.10.1. Tipburn score was taken for individual leaves. Another 5 plants were again selected from each treatment for nutrient analysis. See details on how samples were prepared for nutrient analysis at chapter 3.11.

## 3.8 Experiment 4- Effect of extra Manganese (Mn) in nutrient solution on tip burn in lettuce growing under high irradiance with LED light

In experiment 4, the effect of extra Mn on growth and the occurrence of tip burn under high irradiance with LED light was under study (Table 7). The plants were pre-cultivated as described above. 24 plants were used for each treatment. Extra Mn of 111 ppm was added to a 50littre nutrient solution with a salinity of 2.2 mS $cm^{-1}$ in one chamber. This treatment was compared

with plants in another chamber that had only nutrient solution with a salinity of 2.0 mS $cm^{-1}$ without extra Mn. Water was pumped for one minute 9 times during the photoperiod for both treatments in the first week when the plants were still small. In the second week, the water timer was adjusted to alternate between 1and 2 minutes every 2 hours to the end of the experiment to compensate for the increasing growth.

*Table 7: Details of treatments used in experiment 4. High light + extra Mn (HL/EMn), High light without extra Mn (HL/wEMn)*

| Treatment | Lamp type | Temp, day (°C) | Temp, night (°C) | Relative Humidity (RH) | Photon flux density (µmol $m^{-2}$ $s^{-1}$) | Photoperiod (hr) | Extra Mn ppm (mg/l) |
|---|---|---|---|---|---|---|---|
| LL/EMn | LED | 20 | 18 | 70% | 206 | 16 | 111 |
| LL/wEMn | LED | 20 | 18 | 70% | 206 | 16 | - |

The plants stayed in the chamber for five weeks and the experiment was terminated. 10 plants were randomly sampled from each treatment for tipburn and growth assessment. For details of how growth and tipburn assessment was done, see chapter 3.10.1. Tipburn score was taken for individual leaves. Another 5 plants were again selected from each treatment for nutrient analysis. See details on how samples were prepared for nutrient analysis at chapter 3.11.

## 3.9 Experiment 5- Effects of Manganese (Mn) foliar spray on development of tipburn and growth of lettuce in high and low irradiance provided by LEDs

Four treatments were under study. Table 8 gives details of each treatment. The experiment started with five leaf stage plants. Pre-cultivated was done as described above.

Two chambers, one with low light intensity and one with high light intensity, had 40 plants each. In each chamber, 20 plants were unsprayed, and 20 plants were labeled and sprayed with 15 ml Lebosol ® Mn mixed in 1Littre of water. The Mn solution was sprayed three times during the whole experimental period at 5 days interval (15ml of Mn/1L$H_2O$ three times during the experiment (30/9, 5/10, and 9/10)).

The plants grown in 270 µmol $m^{-2}$ $s^{-2}$ irradiance were watered with nutrient solution for 1 and 2 minutes (alternating) every 2 hours during the photoperiod. The plants grown in 135 µmol $m^{-2}$ $s^{-2}$ were watered with a nutrient solution for 1 minute every second hour during the photoperiod.

*Table 8: Details of treatments used in experiment 1. High light + extra Mn (HL/EMn), High light without extra Mn (HL/wEMn), low light + extra Mn (LL/EMn), low light without extra Mn (LL/wEMn)*

| Treatment | Lamp, type | Photoperiod (hr) | Day, temp (˚C) | Night, temp (˚C) | Photon flux density (µmol $m^{-2}$ $s^{-1}$) | Relative humidity (RH) | Mn spray (Lebosol) |
|---|---|---|---|---|---|---|---|
| HL/EMn | LED | 16 | 20 | 18 | 270 | 70% | + |
| HL/wEMn | LED | 16 | 20 | 18 | 270 | 70% | - |
| LL/EMn | LED | 16 | 20 | 18 | 135 | 70% | + |
| LL/wEMn | LED | 16 | 20 | 18 | 135 | 70% | - |

The plants grew for about three weeks under the different treatments and the experiment ended. Seven samples from each treatment were selected at random for tipburn assessment and growth rate (fresh weight (gr), dry weight, leaf number, and length of longest leaf (cm)). Details of how growth and tipburn assessment were done can be seen at chapter 3.10.1. Individual leaves were assessed for tipburn.

## 3.10 Registrations

At the end of each experiment, samples of plants were taken at random and registered for severity of tipburn, growth, and nutrient analysis.

### 3.10.1 Growth and Severity of tipburn

Registration of tipburn severity was done once for all the experiments. All registrations were done the same day the experiment ended. The plant was cut to the base with scissors without roots. It was then placed on an electronic weighing balance and the fresh weight was taken. The leaves were then separated from the rosette and arranged on a table from the first leaf after the

cotyledon to the last leaf <1cm (Fig 14). The longest leaf of each plant was numbered, and measured with a measuring tape, and the length was recorded as the longest leaf.

Tipburn severity was scored between 0 (no tipburn) and 5 (the whole edge including tips are brown) for each leaf. Completely dead leaves were not scored but counted. The assessment was done according to the scale developed by the NLR (Norwegian Extension Service) (see appendix 1). After scoring, leaves were packed into a paper bag and labeled for drying.

### 3.10.2 Fresh weight (FW) and Dry weight (DW)

The fresh weight of plants was measured on the same day the experiment ended for all the experiments. The whole lettuce plant without the roots was weighed on an electronic weighing balance and the value was recorded as the fresh weight (FW). Assessed leaves (for tipburn severity) were then placed in brown enveloped together with the cotyledon and the stem. This was labeled and kept in the oven at 67°C for at least 7 days for drying (Fig 13). 10 empty bags of the same size as those containing the leaves were also placed in the oven. After drying, the labeled bags containing the plants were weighed on an electronic weighing balance and the values were recorded. The empty bags were also weighed together, and the average was used to find the actual dry weight of the plants without bags (dry weight). This was done by subtracting the average weight of the 10 papers from the weight of the dried sample with the bag to get the actual weight of the plants.

Water content was calculated as the difference between fresh weight and dry weight.

Mathematically represented as

Water content (WC) = DW – FW

% Water content (WC) was calculated as below

% WC = WC/FW *100


*Figure 13: Labelled bags containing the plants (yellow arrow) and labeled tubes containing outer and inner parts of source leaves and sink leaves (blue arrow) for nutrient analysis in the oven at 62°C for 7 days (left). Photo: Gifty Kodua; oven used in drying samples (right). photo: Martin Knoop*

## 3.11 Sampling of leaves for nutrient analysis

From experiments 1, 2, 3, and 4, five plants were randomly selected from each treatment for analysis of Ca, Mg, K, Mn, and Zn accumulation at harvest. Outer and inner parts of 3 old (source) and 3 whole young (sink) leaves were taken. Leaves were separated and spread out on the table. The three old (source) leaves were selected as the first three leaves after the first two leaves not counting the cotyledon and completely dead leaves. In figure 14 below, that will be leaf number 6 to leaf number 8 from the right bottom line.

With the help of small scissors, an outer cut was made about 3 cm from the tip of the leaf. The inner cut was made about 7 cm from the base of the leaf and taking about 3cm of the inner leaf (Figure 15a). The young leaves (Figure 15b) were selected as the three leaves after 5 leaves >1. In figure 14, it will be leaf numbers 7-9 from the upper right.

*Figure 14: Leaves spread on a table to assess tipburn severity. The same way old and young leaves were selected for nutrient analysis. Photo: Gifty Kodua*

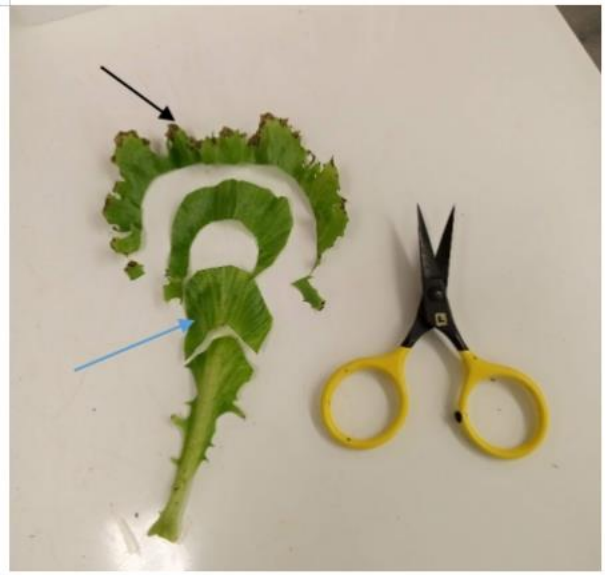


*Figure 15a: Old (source) leaf showing outer leaf (black arrow) and inner leaf (blue arrow) cut outs, to the right of the leaf is the special scissors used. Photo: Gifty Kodua*

*Figure 15b: Three sink leaves selected for nutrient analysis. Photo: Gifty Kodua*

### 3.11.1 Preparation of leaves for nutrient analysis

Selected leaves were kept in labeled 50 ml tubes and dried at 67°C in the oven (Figure 13). After drying, the leaves were grinded in a mortar with a pistil into a fine powder (Figure 16). The powdered leaves were collected into smaller labeled tubes for nutrient analysis. Analysis of the elements (Ca, Mg, K, Mn, Zn) were performed with the powdered samples by the LabTek laboratory (BioSci, NMBU) and measured with ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) method (Greenfield, 1983).

*Figure 16: Showing pictures of mortars with pistils used in grinding the dried leaves and dried leaves in labelled 50ml tubes ready to be grinded for nutrient analysis (left), powdered sample after grinding (right). Photo: Gifty Kodua*

## 3.12 Measurement of Antioxidant capacity using FRAP (Ferric Reducing Antioxidant Power)

Measurement of antioxidant capacity was performed for young leaves of plants from experiment 4 and 5. Five plants were randomly sampled from each treatment. Three young leaves were selected the same way the young leaves were selected for nutrient analysis. The leaves were kept in a labeled 50ml tube and immediately frozen in liquid nitrogen to keep them fresh before storage in -80℃ freezer until usage. Quantitative analysis of the samples for antioxidant potential was performed with OxiSelect Ferric Reducing Antioxidant Power (FRAP) Assay Kit (Cell Biolabs, Inc., CA, USA).

### 3.12.1 Sample preparation and measurement

Frozen samples were grounded in liquid nitrogen to keep their freshness with mortar and pistil. The grounded leaves were kept in labeled tubes and immediately kept in -80℃ freezer until usage. 10 mg of the powdered tissues were weighed into labeled Eppendorf tubes. 1ml cold IX Assay was added to the weighed (10mg) sample, vortex, and centrifuged at 12000rpm for

15minutes at 4℃. The supernatant was collected and transferred into new corresponding labeled Eppendorf tubes and kept on ice. Samples were either tested immediately or stored at -80℃.

Iron (ll) standards and reaction reagent were prepared immediately just before performing the assay. Reaction mixtures were measured into a microplate reader (Biochrom Asys UVM 340 with KIM, UK). Each standard, sample, and control were assayed in duplicate. The absorbance values of the samples were measured against iron (II) standards using 540nm as the primary wavelength.

### 3.13 Statistical analysis

Results were documented and statistically analyzed. Excel spreadsheet was used to treat the raw data before statistical analyses of leaf per day, longest leaf, dry weight, fresh weight, water content, and cation content were performed in Minitab using analysis of variance (One-way ANOVA). Means were separated using Tukey's test at the 5% level of significance. Tipburn graphs were created with an excel spreadsheet. A nonparametric Mann-Whitney test was used to test the effects of irradiance (HPS and LED) on the severity of tipburn (outer leaves, inner leaves, and all leaves) using a p-value of 0.05 for the null hypothesis ($H_0$: $N_1$-$N_2$ = 0). Regression analysis was performed to establish the correlation between inner tipburn in high and low light and cation content in Minitab.

## 4 Results

### 4.1 Experiment 1 - Effect of extra Mn in nutrient solution on growth and tipburn incidence of lettuce in moderate and high irradiance provided by HPS

Increased irradiance from 150 µmol $m^{-2}$ $s^{-1}$ to 300µmol $m^{-2}$ $s^{-1}$ induces severe inner and outer tipburn (Knoop, 2019). This experiment was aimed at assessing the effect of extra manganese (111ppm) in nutrient solution on growth parameters, occurrence, and severity of tipburn in frillice lettuce under an HPS lamp with high (300 µmol $m^{-2}$ $s^{-1}$) and moderate (150 µmol $m^{-2}$ $s^{-1}$)

irradiance. It was also of interest to know if inner leaf and outer leaf tipburn have a connection with the amount of Ca, Mg, K, Mn, and Zn in those areas.

All the plants grew in moderate irradiance (150 µmol $m^{-2}$ $s^{-1}$) for 18 hours and were watered with a normal nutrient solution in both treatments for two weeks before the nutrient solution in both chambers was changed to a nutrient solution with extra Mn and in one of the chambers, the irradiance was increased to 300 µmol $m^{-2}$ $s^{-1}$. The set-up was organized like this to test if increased Mn in the nutrient solution would reduce the risk for tipburn of lettuce in increased irradiance.

### 4.1.1 Growth

A significant difference in all growth parameters measured between the two treatments was found (Table 9). Plants growing with extra Mn in moderate irradiance (150 µmol $m^{-2}$ $s^{-1}$) induced an average of 0.9 more leaves per day than plants grown in high irradiance. Moderate irradiance induced longer leaves and the longest leaf was on average 3.12 cm longer compared to high irradiance (Table 9). Increased irradiance gave a higher fresh weight (22.8%) and dry weight (46.2%) compared to plants in moderate light treatment. Moderate irradiance (150 µmol $m^{-2}$ $s^{-1}$) gave significantly higher water content than high irradiance (Table 9).

*Table 9. Effect of extra Mn in the nutrient solution on growth parameters of frillice lettuce grown in moderate (150 µmol $m^{-2}$ $s^{-1}$) and high (300 µmol $m^{-2}$ $s^{-1}$) irradiance using HPS as the light source. The plants were grown at 20℃ day and 18℃ night temp with 70% RH for 28 days. The ANOVA results give the means, standard error (SE), and P-value of the treatments. HPS+Mn= HPS lamp with extra Mn in the nutrient solution. A Turkey test was used to compare the means of the treatments. Different letters in each column are significantly different at 5% level. In each treatment, N=10.*

| Treatments | Leaf per day | Longest leaf *cm* | Fresh weight *(g per plant)* | Dry weight *(g per plant)* | Water content *(%)* |
|---|---|---|---|---|---|
| HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$) | 1.09 A ±0.04 | 19.80 A ± 1.05 | 145.59 B ± 16.51 | 7.04 B ± 1.00 | 95.18 A ± 0. |
| HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$) | 1.00 B ± 0.07 | 16.68 B ± 0.85 | 178.83 A ± 14.95 | 10.29 A ± 1.00 | 94.25 B ±0.32 |
| ***P-value*** | ***0.003*** | ***<0.001*** | ***<0.001*** | ***< 0.001*** | ***<0.001*** |

### 4.1.2 Tipburn assessment

Results from the tipburn assessment showed no significant difference in average tipburn severity for all leaves between treatments (p=0.850). From the line graph, both HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$) and HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$) treatments resulted in decreasing severity from the outermost leaf to the innermost leaf (Figure 17a). Plants in moderate light with extra Mn in nutrient solution gave a slightly higher tipburn score in outer/older leaves (Figure 17b) but no significant difference was found between treatments (p-value = 0.545). A significantly higher tipburn severity was found in the inner leaves of plants in moderate light with extra Mn in nutrient solution compared with high light irradiance (p-value = 0.005) (Figure 17b).

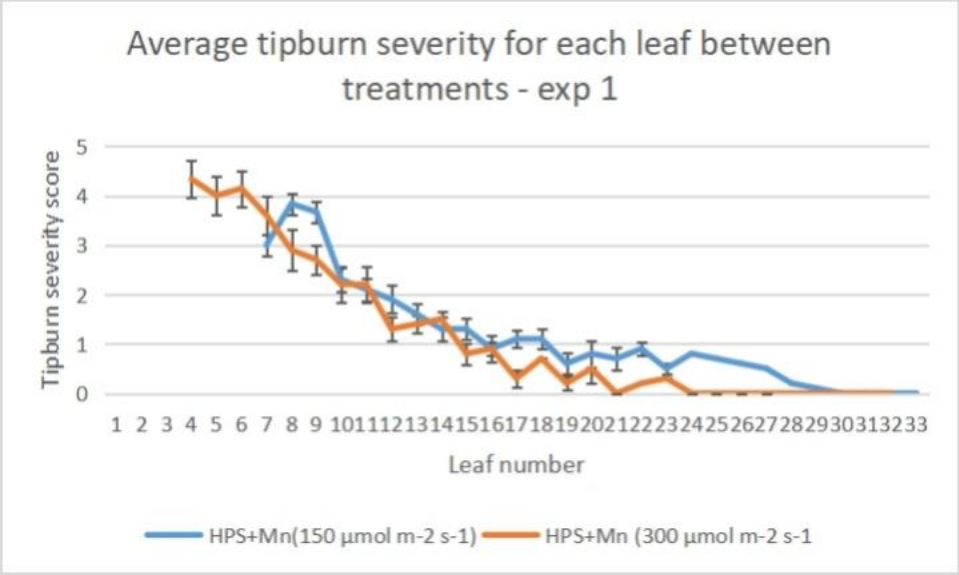



*Figure 17a: Average tipburn score (0-5) (± SE) between plants growing with extra Mn in nutrient solution under moderate light with HPS = HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$) and extra Mn in nutrient solution under high light with HPS= HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$) for each leaf for experiment 1. The first leaf which in this case is leaf number 4 is the first outer leaf after the cotyledon and completely dead leaves. A tipburn score of 5 is the highest form of severity while 1 is the least severe, and 0 is no tipburn.* N=10.

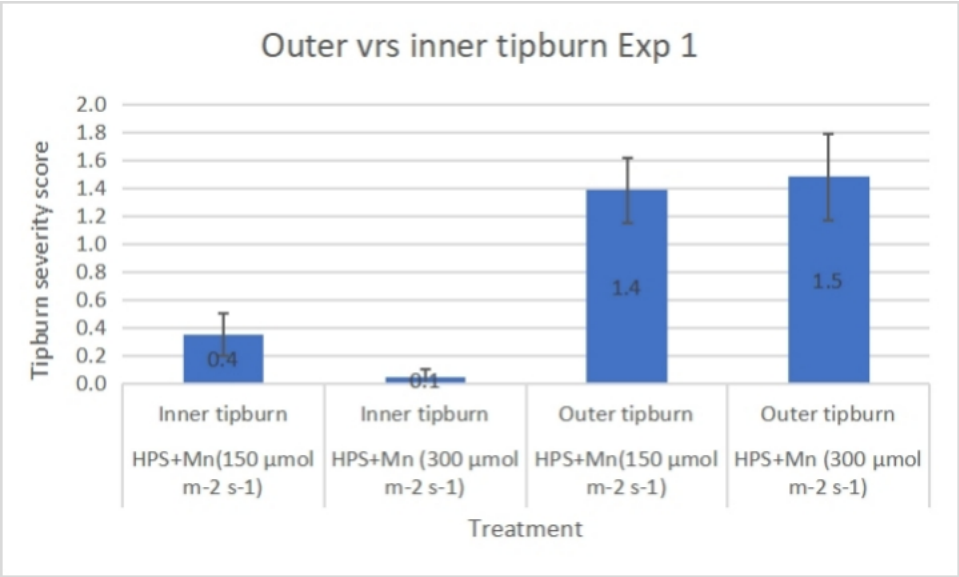



*Figure 17b: Average tipburn severity score (0-5) (± SE) between outer and inner leaves of plants growing with extra Mn in nutrient solution under moderate light with HPS = HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$) and extra Mn in nutrient solution under high light with HPS= HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$) for experiment 1. P-value for outer tipburn = 0.545 and inner tipburn = 0.005. N = 10. A tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

### 4.1.3 Cation accumulation

Significantly higher calcium content was found in young leaves of plant samples from moderate light compared to high light treatment (p = 0.019). The edges and inner parts of old leaves did not show any significant difference in calcium content between treatments. No significant differences in magnesium and potassium content were found between treatments, either in young leaves, nor edges, and inner parts of old leaves (Table 10).

Significant higher manganese content was found in young leaves and edges of old leaves of moderate irradiance treatment compared with high irradiance (p-value = 0.017 and 0.001 respectively). There was no significant difference in manganese content in the inner part of old leaves between treatments although a higher content was found in moderate irradiance treatment

(Table 10). For Zinc, a significantly higher content was found in young leaves of moderate light. In the edges and inner parts of old leaves, plants in moderate irradiance had higher content, but it was not significant (Table 10).

*Table 10: Effect of extra Mn in the nutrient solution on cation accumulation in young leaves, edge* of old leaves, *and inner parts of old leaves of frillice lettuce grown in moderate (150 µmol $m^{-2}$ $s^{-1}$) and high (300 µmol $m^{-2}$ $s^{-1}$) irradiance using HPS as a light source. P-value, mean and standard error (SE) for the content of calcium (Ca) potassium (K), and magnesium (Mg) in % and manganese (Mn) and zinc (Zn) in mg/kg. Turkey test was used to compare the means of the treatments. Different letters in each column for each nutrient are significantly different at 5% level. In each treatment, N=5.*

| Treatment | Element | | |
|---|---|---|---|
| | *Ca (%)* | | |
| | Young | Old edge | Old inner |
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 0.30 B ±0.11 | 1.72 A ± 0.26 | 1.36 A ± 0.11 |
| **HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$)** | 0.46 A ± 0.05 | 1.75 A ± 0.08 | 1.31 A ± 0.22 |
| *P value* | *0.019* | *0.812* | *0.691* |
| | *Mg (%)* | | |
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 0.16 A ± 0.03 | 0.83 A ± 0.11 | 0.28 A ± 0.01 |
| **HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$)** | 0.21 A ± 0.04 | 0.82 A ± 0.10 | 0.27 A ±0.02 |
| *P value* | *0.065* | *0.816* | *0.135* |
| | *K (%)* | | |
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 3.62 A ± 0.47 | 6.28 A ± 0.63 | 12.79 A ± 0.97 |
| **HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$)** | 4.28 A ± 0.53 | 6.50 A ± 0.74 | 13.31 A± 0.71 |
| *P value* | *0.070* | *0.625* | *0.371* |
| | *Mn (mg/kg)* | | |
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 1012 B ± 354 | 5730 B ± 939 | 2158.8 A±169.3 |
| **HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$)** | 1504.2 A ± 90.0 | 8163 A ± 419 | 2230.5 A± 121 |
| *P value* | *0.017* | *0.001* | *0.463* |
| | *Zn (mg/kg)* | | |

| | | | |
|---|---|---|---|
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 48.95 B ± 9.86 | 45.06 A ± 15.12 | 32.00 A± 8.86 |
| **HPS+Mn (150 µmol $m^{-2}$ $s^{-1}$)** | 74.54 A ± 11.23 | 58.88 A ± 12.72 | 35.22 A ±7.35 |
| *P value* | *0.005* | *0.156* | *0.550* |

## 4.2 Experiment 2 - Extra Manganese (Mn) in nutrient solution on growth and tipburn of lettuce grown in high irradiance provided by HPS lamps

The aim was to compare and assess the effect of adding extra Mn in nutrient solution with control (without extra Mn) when plants are moved from moderate (150 µmol $m^{-2}$ $s^{-1}$) to high (300 µmol $m^{-2}$ $s^{-1}$) irradiance under an HPS lamp on plants growth and the occurrence and severity of tipburn in frillice lettuce. The high light (300 µmol $m^{-2}$ $s^{-1}$) treatment was given to the plants (both treatments) in the last two weeks of their growth unlike in experiment 1 where the light was increased in one of the chambers. Other environmental factors were the same in both chambers.

### 4.2.1 Growth

Treated plants (HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$) gave higher fresh weight and dry weight (30.6% and 19.7% respectively) than the control (Table 11). Treatment with extra Mn in nutrient solution did not significantly affect leaf unfolding rate/leaf per day compared with the control (HPS-Mn (300 µmol $m^{-2}$ $s^{-1}$)). Although the average longest leaf was recorded in treatment with extra Mn in nutrient solution with high light, it was not significantly different from the control, (p=0.213). There was no significant difference in water content (P-value= 0.069) between the treatment (Table 11).

*Table 11. Effect of extra Mn on growth parameters of frillice lettuce when plants are moved from low to high irradiance using HPS lamp. The plants were grown at 20°C day and 18°C night temp with 70% RH for 28 days. HPS+Mn= HPS lamp, extra manganese in nutrient solution; HPS-Mn= HPS lamp, without extra Mn in the nutrient solution. The ANOVA results give the means, standard error (SE), and P-value of the treatments. Turkey test is used to compare the means of the treatments. Different letters in each column are significantly different at 5% level. In each treatment, N=10.*

| Treatment | Leaf per day | Longest leaf | Fresh weight | Dry weight | Water content |
|---|---|---|---|---|---|
| | | *cm* | *(g per plant)* | *(g per plant)* | *(%)* |
| HPS-Mn (300 µmol $m^{-2}$ $s^{-1}$) | 1.08 A ± 0.05 | 8.60 A ± 0.52 | 137.95 B ± 14.55 | 9.23 B ± 0.63 | 93.25 A ± 0.79 |
| HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$) | 1.08 A ± 0.06 | 9.30 A ± 1.64 | 180.22 A ± 12.86 | 11.05 A ± 1.60 | 93.89 A ± 0.65 |
| ***P-value*** | ***0.791*** | ***0.213*** | ***<0.001*** | ***0.004*** | ***0.069*** |

### 4.2.2 Tipburn assessment

There was found a significant difference between treatments (p-value = 0.021) with extra Mn significantly reducing the average tipburn severity for individual leaves compared to control (Figure 18a). Treatment with the normal nutrient solution showed increased tipburn severity in both outer leaves and inner leaves compared with plants growing with extra Mn in nutrient solution (p=0.002 and 0.037 respectively) (Figure 18b).

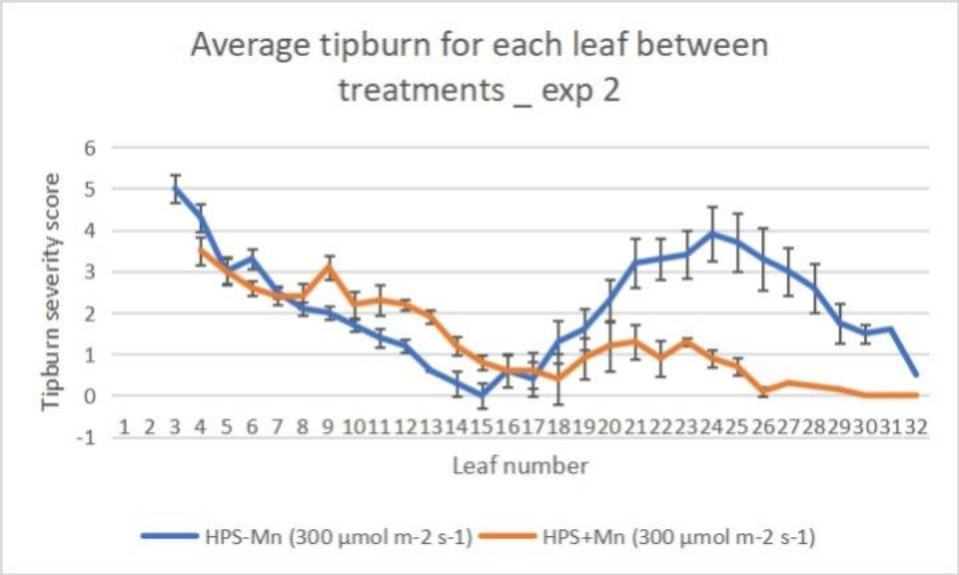



*Figure 18a: Average tipburn score (0-5) (± SE) between plants growing with the normal nutrient solution under high light with HPS = HPS-Mn (300 µmol $m^{-2}$ $s^{-1}$) and extra Mn in nutrient solution under high light with HPS=*

*HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$) on each leaf for experiment 2. The first leaf, which in this case is 3, is the first outer leaf after the cotyledon and completely dead leaves. A tipburn score of 5 is the highest form of severity while 1 is the least severe, and 0 is no tipburn.*

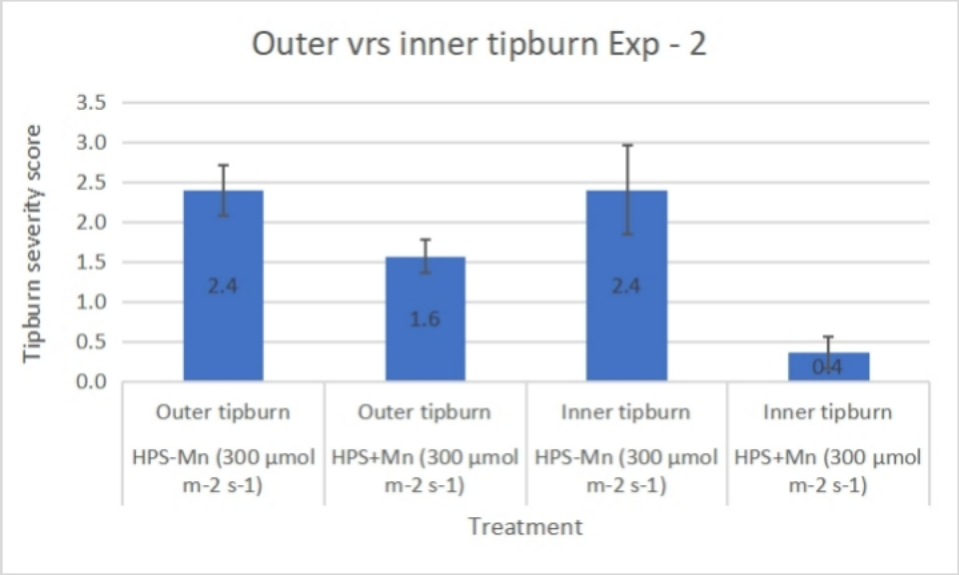



*Figure 18b: Average tipburn severity score (0-5) (± SE) between outer and inner leaves of plants growing with the normal nutrient solution under high light with HPS = HPS-Mn (300 µmol $m^{-2}$ $s^{-1}$) and extra Mn in nutrient solution under high light with HPS= HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$) for experiment 2. P-value for outer tipburn = 0.037 and inner tipburn = 0.002. N = 10. A tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

### 4.2.3 Cation accumulation

There was no significant difference in calcium (Ca) content in young leaves, edge of old leaves, and inner parts of old leaves between treatments. Significantly higher magnesium (Mg) content was found in the edge of old leaves of control than treatment with extra Mn in the nutrient solution. For young leaves and the inner part of old leaves, there was no significant difference found in the magnesium content between treatments (Table 12).

Higher accumulation of potassium (K) was found in control compared with treatment with extra Mn in the nutrient solution, but the difference was not significant at 5% mean level (Table 12). For manganese, treatment with extra Mn in nutrient solution resulted in significantly higher accumulation in young leaves, edge of old leaves, and inner parts of old leaves compared with control. P = < 0.001. Zinc content in plant samples from both treatments was below detection level (Table 12).

*Table 12: Effect of extra Mn in the nutrient solution (HPS+Mn, 300 µmol $m^{-2}$ $s^{-1}$) compared with control (HPS-Mn, 300 µmol $m^{-2}$ $s^{-1}$) on cation accumulation in young leaves, edge of old leaves, and inner part of old leaves of frillice lettuce when plants are moved from low to high irradiance under HPS lamp. The plants were harvested after 28 days in high irradiance. P-value, mean and standard error (SE) for the content of calcium (Ca) potassium (K) and magnesium (Mg) in % and manganese (Mn) and zinc (Zn) in mg/kg. Turkey test was used to compare the means of the treatments. Different letters in each column for each nutrient is significantly different at 5% level. In each treatment, N=5.*

| Treatment | Element | | |
|---|---|---|---|
| | *Ca (%)* | | |
| | Young | Old edge | Old inner |
| **HPS-Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 0.21 A ± 0.04 | 1.66 A ± 0.10 | 1.16 A ± 0.15 |
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 0.20 A ± 0.04 | 1.61 A ± 0.09 | 1.22 A ± 0.16 |
| *P value* | *0.762* | *0.443* | *0.564* |
| | *Mg (%)* | | |
| **HPS-Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 0.13 A ± 0.03 | 1.00 A ± 0.14 | 0.28 A ± 0.09 |
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 0.12 A ± 0.01 | 0.72 B ± 0.08 | 0.38 A ± 0.07 |
| *P value* | *0.299* | *0.015* | *0.078* |
| | *K (%)* | | |
| **HPS-Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 3.46 A ± 0.46 | 6.70 A ± 0.67 | 11.38 A ±1.74 |
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 3.37 A ± 0.11 | 5.78 A ± 0.86 | 11.29 A ± 0.69 |
| *P value* | *0.683* | *0.098* | *0.915* |
| | *Mn (%)* | | |

| | | | |
|---|---|---|---|
| **HPS-Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 14.95 B ± 3.55 | 194.7 B ± 25.8 | 34.27 B ± 4.90 |
| **HPS+Mn (300 µmol $m^{-2}$ $s^{-1}$)** | 665.4 A ± 134.6 | 4421 A ± 485 | 2072 A ± 263 |
| *P value* | *<0.001* | *<0.001* | *<0.001* |

## 4.3 Experiment 3 - Effect of extra Manganese (Mn) in nutrient solution on growth and tipburn of lettuce grown in low irradiance provided by LED lamps

Experiment 3 was aimed at testing the effect of extra Mn in nutrient solution on the occurrence and severity of tipburn, and growth parameters of frillice lettuce growing in low (134 µmol $m^{-2}$ $s^{-1}$) irradiance with LED light compared with the control (without extra Mn). Measurement of cations (Ca, Mg, K, Mn, and Zn) was also performed on young leaves, edges, and inner part of old leaves of plant samples from both treatments to find the relationship between tipburn incidence and cation accumulation. Finally, FRAP analysis was performed to test the antioxidant capacity of plant samples from both treatments.

### 4.3.1 Growth results

There was a significant difference in leaf unfolding rate between the treatments. The control had in average 0.8 more leaves per day than the treatment with extra Mn. Leaf length was not significantly different between the treatment (p=0.277). Also, there was also no significant difference found in fresh weight although the control (LED-Mn (134 µmol $m^{-2}$ $s^{-1}$) recorded a higher mean. There was a significant difference in dry weight with LED-Mn (134 µmol $m^{-2}$ $s^{-1}$) having a higher dry weight than LED+Mn (134 µmol $m^{-2}$ $s^{-1}$) (p=0.013). Higher water content was found in treatment with extra Mn (Table 13).

*Figure 19: Lettuce plants at the end of experiment 3. Treated with extra manganese in nutrient solution (left) and treated with normal nutrient solution (right). Photo: Sissel Torre*

*Table 13: Effect of extra Mn in nutrient solution on growth parameters of frillice lettuce growing in low irradiance with LED as the light source. The plants were grown at 20℃ day and 18℃ night temp with 70% RH for 22 days. LED+Mn= LED lamp, extra manganese in nutrient solution; LED-Mn= LED lamp, without extra Mn in the nutrient solution. The ANOVA results give the means, standard error (SE), and P-value of the treatments. Turkey test is used to compare the means of the treatments. Different letters in each column are significantly different at 5% level. In each treatment, N=10.*

| Treatment | Leaf per day | Longest leaf *cm* | Fresh weight *(g per plant)* | Dry weight *(g per plant)* | Water content *(%)* |
|---|---|---|---|---|---|
| LED+Mn (134 µmol $m^{-2}$ $s^{-1}$) | 1.01 B ± 0.06 | 16.20 A ± 0.99 | 104.02 A ± 10.06 | 4.10 B ± 0.35 | 96.05 A ± 0.16 |
| LED-Mn (134 µmol $m^{-2}$ $s^{-1}$) | 1.09 A ± 0.08 | 15.61 A ± 1.34 | 110.20 A ± 13.79 | 4.67 A± 0.52 | 95.75 B± 0.31 |
| ***P-value*** | ***0.002*** | ***0.277*** | ***0.268*** | ***0.010*** | ***0.013*** |

### 4.3.2 Tipburn assessment

A significantly lower average tipburn score was recorded for treatment with extra Mn in nutrient solution (LED+Mn) when all leaves were assessed compared to control (LED-Mn) ($p=0.004$). Both treatments showed a higher tipburn score in their outer leaves compared to inner when individual leaves were assessed (Figure 20a). Treatment with extra Mn in the nutrient solution (LED+Mn) gave a sharp reduction in tipburn severity approaching leaves in the middle of the plants when individual leaves were assessed (Figure 20a). Treatment with extra Mn in the nutrient solution (LED+Mn) resulted in lower average outer tipburn and no inner tipburn compared with when plants were given normal nutrient solution (LED+Mn). (Figure 20b).

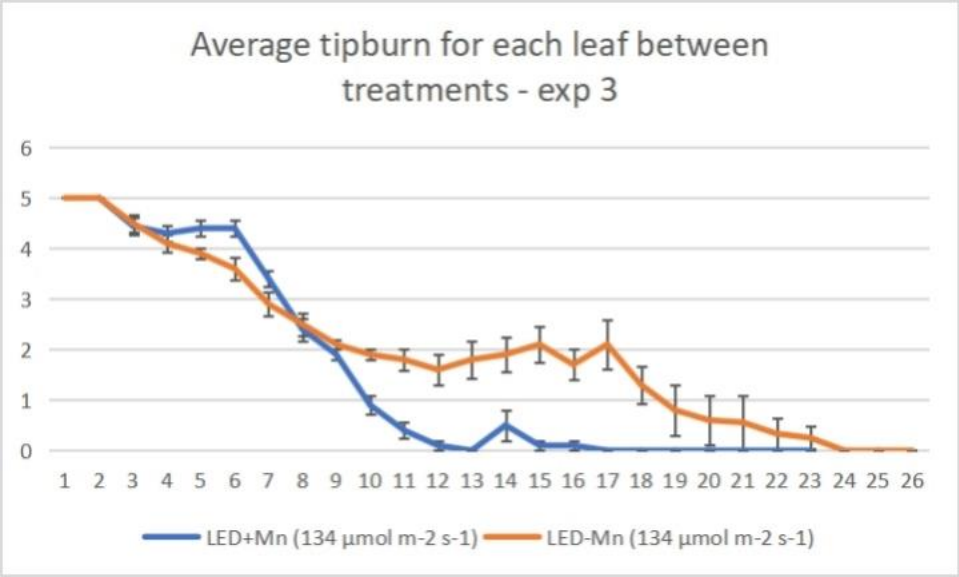



*Figure 20a: Average tipburn score (0-5) (± SE) between plants growing with extra Mn in nutrient solution under moderate light with LED = LED+Mn (134 µmol $m^{-2}$ $s^{-1}$) and normal nutrient solution under moderate light with LED= LED-Mn (134 µmol $m^{-2}$ $s^{-1}$) on each leaf for experiment 3. Leaf 1 is the first outer leaf after the cotyledon. Tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

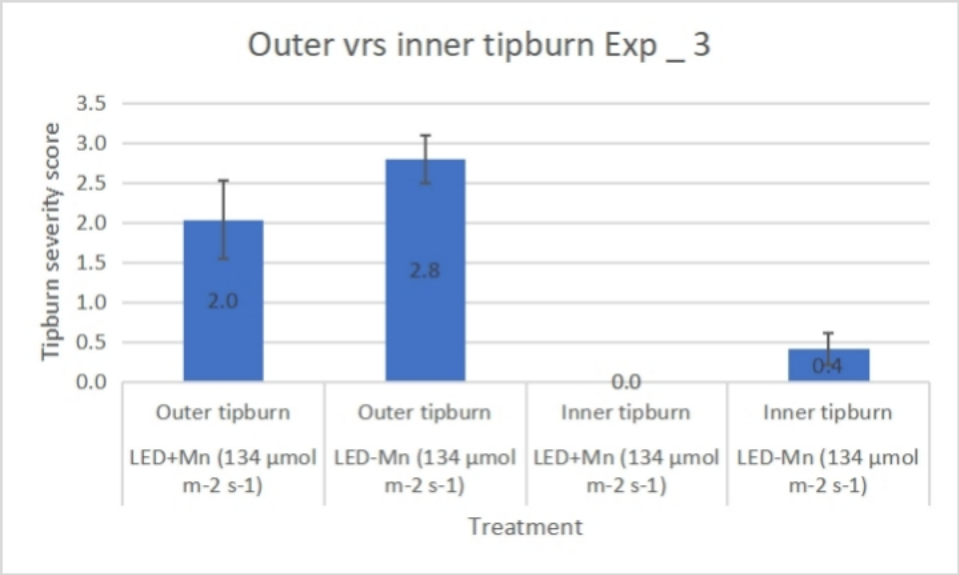



*Figure 20b: Average tipburn severity score (0-5) (± SE) between outer and inner leaves of plants growing with extra Mn in nutrient solution under moderate light with LED = LED+Mn (134 µmol $m^{-2}$ $s^{-1}$) and normal nutrient solution under moderate light with LED= LED-Mn (134 µmol $m^{-2}$ $s^{-1}$) for experiment 3. P-value for outer tipburn = 0.007. N = 10. A tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

### 4.3.3 Cation accumulation

There was significantly higher Ca content in young leaves of treatment with extra Mn while the edges of old leaves and inner part of old leaves did not result in any significant difference between treatments (Table 14). Magnesium (Mg) content was low in young leaves, edges of old leaves, and inner part of old leaves of treatment with extra manganese in nutrient solution (LED+Mn) compared with the control (Table 14).

There was a significantly lower potassium content in young leaves of treatment with extra manganese in nutrient solution (LED+Mn) compared with the control. In the edges and inner part of old leaves, potassium content was high but not significant at 5% level when extra manganese was added to the nutrient solution compared with the control. Significant higher manganese accumulated in young leaves, edges of old leaves, and inner part of old leaves of treatment with extra manganese in nutrient solution (LED+Mn) compared with the control (Table 14).

For Zn, the highest content was found in young leaves of plants from treatment with extra Mn compared with control. This was significant at the 5% level (Table 14). Accumulation of Zn in the edges of old leaves and the inner part of old leaves in both treatments were below detection level.

*Table 14: Effect of extra Mn in the nutrient solution on cation accumulation in young leaves, edge of the old leaf (old tip), and inner part of old leaves (old inner) of frillice lettuce growing in low irradiance (134 µmol $m^{-2}$ $s^{-1}$) with LED as the light source. P-value, mean and standard error (SE) for the content of calcium (Ca) potassium (K), and magnesium (Mg) in % and manganese (Mn) and zinc (Zn) in mg/kg. Turkey test was used to compare the means of the treatments. Different letters in the same column for each nutrient are significantly different at 5% level. In each treatment, N=5.*

| Treatment | Element | | |
|---|---|---|---|
| | Ca (%) | | |
| | Young | Old edge | Old inner |
| **LED+Mn (134 µmol $m^{-2}$ $s^{-1}$)** | 0.38 A ±0.03 | 1.57 A ± 0.07 | 1.23 A ± 0.12 |
| **LED-Mn (134 µmol $m^{-2}$ $s^{-1}$)** | 0.32 B ± 0.03 | 1.53 A ± 0.11 | 1.29 A ± 0.11 |
| *P value* | *0.011* | *0.478* | *0.460* |
| | *Mg (%)* | | |
| **LED+Mn (134 µmol $m^{-2}$ $s^{-1}$)** | 0.16 B ± 0.01 | 0.67 B ± 0.04 | 0.29 B ± 0.04 |
| **LED-Mn (134 µmol $m^{-2}$ $s^{-1}$)** | 0.24 A ± 0.02 | 0.79 A ± 0.04 | 0.39 A ± 0.04 |
| *P value* | *<0.001* | *0.001* | *0.003* |
| | *K (%)* | | |
| **LED+Mn (134 µmol $m^{-2}$ $s^{-1}$)** | 4.90 B ± 0.37 | 5.89 A ± 0.45 | 12.37 A ± 0.32 |
| **LED-Mn (134 µmol $m^{-2}$ $s^{-1}$)** | 5.55 A ± 0.49 | 5.51 A ± 0.55 | 12.01 A ± 0.63 |
| *P value* | *0.042* | *0.257* | *0.280* |
| | *Mn (mg/kg)* | | |
| **LED+Mn (134 µmol $m^{-2}$ $s^{-1}$)** | 1,054.3 A ± 98.3 | 3706 B ± 323 | 1830.3 B ± 192.8 |
| **LED-Mn (134 µmol $m^{-2}$ $s^{-1}$)** | 30.54 B ± 3.05 | 168.36 A ± 14.06 | 40.71 A ± 3.48 |
| *P value* | *<0.001* | *<0.001* | *<0.001* |

| | *Zn (mg/kg)* |
|---|---|
| LED+Mn (134 µmol $m^{-2}$ $s^{-1}$) | 43.46 A ± 3.39 |
| LED-Mn (134 µmol $m^{-2}$ s | 31.32 B ± 3.91 |
| *P value* | *0.001* |

## 4.4 Experiment 4 - Effect of extra Manganese (Mn) in nutrient solution on growth and tipburn of lettuce grown in high irradiance provided by LED lamps

The aim of the experiment was to further assess how extra manganese (Mn) in nutrient solution affects the growth, occurrence, and severity of tip burn of frillice lettuce growing but this time, in high (206 µmol $m^{-2}$ $s^{-1}$) irradiance using the same LED lamp used in experiment three. This was compared with control that did not get extra Mn in the nutrient solution. Also, measurement of cations (Ca, Mg, K, Mn, and Zn) was performed on young leaves, edge of old leaves, and inner part of old leaves of plant samples from both treatments to find the relationship between tipburn incidence and cation accumulation. Finally, FRAP analysis was performed on young leaves to test the antioxidant capacity of plant samples from both treatments and how it influenced tipburn occurrence in lettuce plants.

By day 13, plants in both treatments had developed a lot of tipburn in their inner leaves (Figure 21). Physical observation of plants at the end of the experiment showed recovery of plants from tipburn in their inner leaves in treatment with extra manganese whilst plants that are grown with the normal nutrient solution still had tipburn in their inner leaves (Figure 22).

### 4.4.1 Growth

There was a significant difference in all growth parameters measured between the treatments (Table 7). Higher values were recorded for leaf per day (1.9), leaf length (1.02 cm), fresh weight (33.1%), and dry weight (18.2%) for the control (LED-Mn (206 µmol $m^{-2}$ $s^{-1}$)) compared with the treatment with extra Mn. A significant higher water content was found in plants growing

without extra Mn (LED-Mn (206 µmol $m^{-2}$ $s^{-1}$)) in nutrient solution compared with the control (Table 15).

*Figure 21: Lettuce plants at 13 days in the growth chamber. Treated with normal nutrient solution (left) and treated with extra manganese in nutrient solution (right). Photo: Gifty Kodua*

*Figure 22: Lettuce plants at the end of experiment 4. Treated with normal nutrient solution (left) and treated with extra manganese in nutrient solution (right). Photo: Gifty Kodua*

*Table 15: Effect of extra Mn in nutrient solution on growth parameters of frillice lettuce growing in moderate* (206 µmol $m^{-2}$ $s^{-1}$) *irradiance with LED as a light source. The plants were grown at 20ºC day and 18ºC night temp with 70% RH for 35 days. LED+Mn= LED lamp, extra manganese in nutrient solution; LED-Mn= LED lamp, without extra Mn in the nutrient solution. The ANOVA results give the means, standard error (SE), and P-value of the treatments. Turkey test is used to compare the means of the treatments. Different letters in each column are significantly different at 5% level. In each treatment, N=10.*

| Treatment | Leaf per day | Longest leaf | Fresh weight | Dry weight | Water content |
|---|---|---|---|---|---|
| | | *cm* | *(g per plant)* | *(g per plant)* | *(%)* |
| LED+Mn (206 µmol $m^{-2}$ $s^{-1}$) | 0.66 B ± 0.05 | 14.13 B ± 0.98 | 85.81 B ± 13.99 | 5.22 B ± 0.62 | 93.88 A ± 0.36 |
| LED-Mn (206 µmol $m^{-2}$ $s^{-1}$) | 0.72 A ± 0.04 | 15.11 A ± 0.67 | 114.19 A ± 13.55 | 6.17 A ± 0.50 | 94.56 B ± 0.40 |
| ***P-value*** | ***0.017*** | ***0.018*** | ***< 0.001*** | ***0.001*** | ***0.001*** |

### 4.4.2 Tipburn assessment

Average tipburn severity on all leaves between treatments showed lower severity for tipburn in treatment with extra Mn in nutrient solution compared with when only normal nutrient solution is given to the plants. This was significant at a p-value <0.001.

Both treatments resulted in a high outer tipburn score, but treatment with normal nutrient solution (LED-Mn, 206 µmol $m^{-2}$ $s^{-1}$) showed a bit higher value (Figure 23a). Average tipburn severity on all outer leaves resulted in lower severity in treatment with extra Mn in nutrient solution (p=0.002) Figure 23b. Treatment with extra Mn in nutrient solution was found to reduce inner tipburn to almost zero compared with when only normal nutrient solution is given to the plants, p-value <0.001 (Figure 23b).

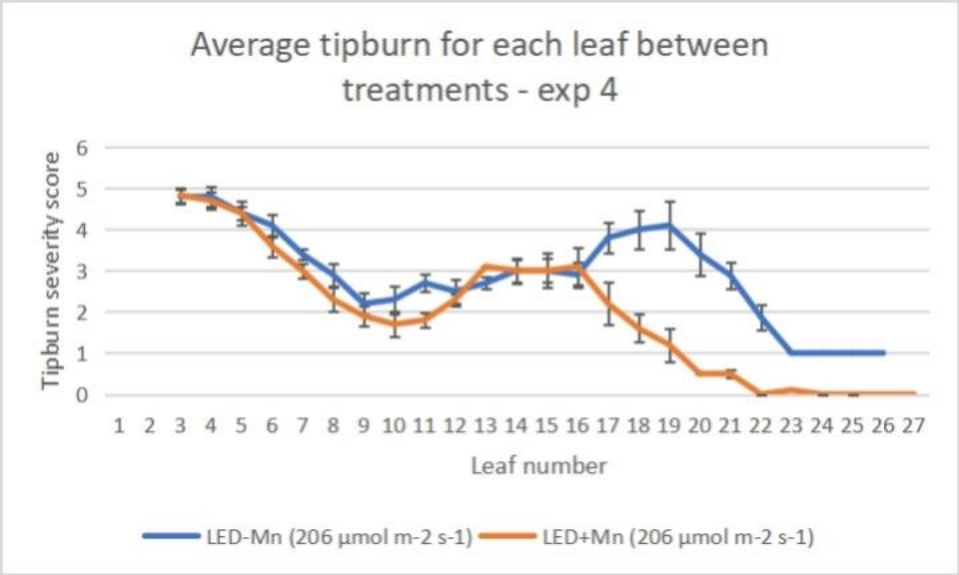



*Figure 23a: Average tipburn score (0-5) (± SE) between plants growing with the normal nutrient solution under moderate light with LED = LED-Mn (206 µmol $m^{-2}$ $s^{-1}$) and extra Mn in nutrient solution under moderate light with LED= LED+Mn (206 µmol $m^{-2}$ $s^{-1}$) for each leaf for experiment 4. The first leaf which in this case is 3 is the first outer leaf after the cotyledon and completely dead leaves. A tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

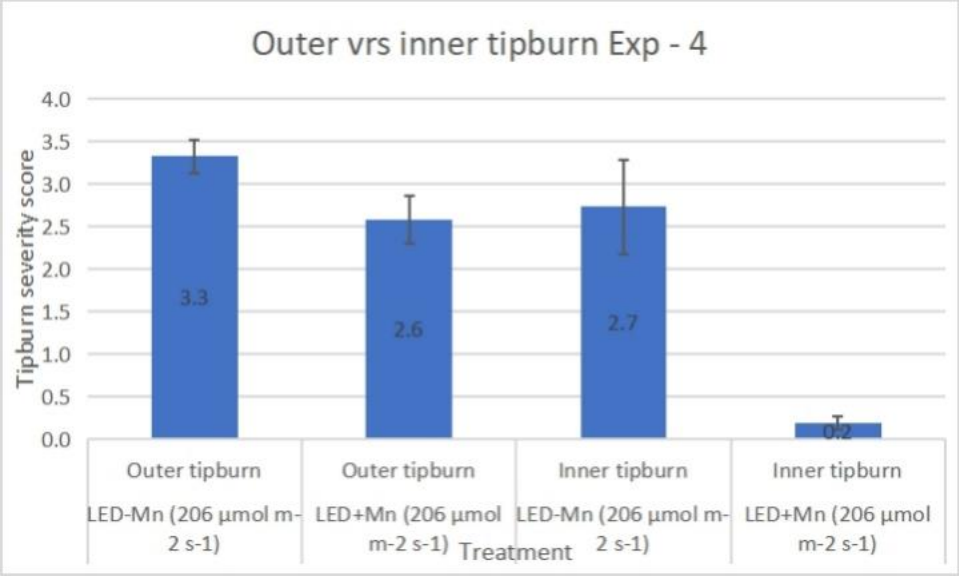



*Figure 23b: Average tipburn severity score (0-5) (± SE) between outer and inner leaves of plants growing with the normal nutrient solution under moderate light with LED = LED-Mn (206 µmol $m^{-2}$ $s^{-1}$) and extra Mn in nutrient*

*solution under moderate light with LED= LED+Mn (206 µmol $m^{-2}$ $s^{-1}$) for experiment 4. P-value for outer tipburn = 0.002 and inner tipburn = < 0.001. N = 10. A tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

### 4.4.3 Cation accumulation

There was no significant difference in calcium content in young leaves of plants between treatments neither was found a significant difference in the inner part of old leaves of plants between treatments. However, high content of calcium was found in the edges of old leaves of plants in extra Mn treatment compared with those without extra Mn treatment (Table 16).

Magnesium content was high in young leaves and the edges of old leaves of plants without extra Mn treatment which was significant at 5% level, but no significant difference was found in the inner part of old leaves of plants between treatments. There was not found any significant difference in potassium content for young leaves, edges of old leaves, and inner parts of old leaves between treatments. For manganese, high content was found in young leaves, edges of old leaves, and inner parts of old leaves of extra Mn treatment compared with without extra Mn treatment (Table 16). Zinc content in plant samples from both treatments was below detection level.

*Table 16: Effect of extra Mn in the nutrient solution on cation accumulation in young leaves, edge of the old leaf (old tip), and inner part of the old leaf (old inner) of frillice lettuce growing in moderate* (206 µmol $m^{-2}$ $s^{-1}$) *irradiance with LED as a light source. P-value, mean and standard error (SE) for the content of calcium (Ca) potassium (K), and magnesium (Mg) in % and manganese (Mn) and zinc (Zn) in mg/kg. Turkey test was used to compare the means of the treatments. Different letters in each column for each nutrient are significantly different at 5% level. In each treatment, N=5.*

| Treatment | Element | | |
|---|---|---|---|
| | *Ca (%)* | | |
| | Young | Old edge | Old inner |
| **LED-Mn (206 µmol $m^{-2}$ $s^{-1}$)** | 0.28 A ± 0.03 | 1.44 B ± 0.10 | 1.08 A ± 0.12 |
| **LED+Mn (206 µmol $m^{-2}$ $s^{-1}$)** | 0.26 A ± 0.07 | 1.61 A ± 0.13 | 1.36 A ± 0.48 |
| *P value* | *0.595* | *0.050* | *0.231* |

| | | | |
|---|---|---|---|
| | *Mg (%)* | | |
| **LED-Mn (206 µmol $m^{-2}$ $s^{-1}$)** | 0.16 A ± 0.01 | 0.68 A ± 0.05 | 0.24 A ± 0.05 |
| **LED+Mn (206 µmol $m^{-2}$ $s^{-1}$)** | 0.13 B ± 0.01 | 0.53 B ± 0.06 | 0.23 A ± 0.04 |
| *P value* | *0.001* | *0.003* | *0.605* |
| | *K (%)* | | |
| **LED-Mn (206 µmol $m^{-2}$ $s^{-1}$)** | 4.04 A ±0.38 | 5.21 A ± 0.56 | 10.78 A±1.25 |
| **LED+Mn (206 µmol $m^{-2}$ $s^{-1}$)** | 4.46 A ±0.38 | 5.70 A ±0.27 | 11.58 A±0.32 |
| *P value* | *0.119* | *0.119* | *0.202* |
| | *Mn (mg/Kg)* | | |
| **LED-Mn (206 µmol $m^{-2}$ $s^{-1}$)** | 41.05 B± 6.37 | 350.2 B± 71.4 | 124.9 B±22.5 |
| **LED+Mn (206 µmol $m^{-2}$ $s^{-1}$)** | 888 A ±258 | 4096 A ± 1559 | 2136 A±249 |
| *P value* | *<0.001* | *0.001* | *<0.001* |

## 4.5 Experiment 5 - Effect of Foliar spray of Mn on growth and tipburn of lettuce grown in high and low irradiance provided by LEDs

Experiment 5 was aimed at evaluating the effect of manganese (Mn) foliar spray on the development and severity of tip burn in high (270 µmol $m^{-2}$ $s^{-1}$) and low (134 µmol $m^{-2}$ $s^{-1}$) irradiance with LED lamp. It was also to assess how the foliar spray affects the growth of the plants. The two chambers with high and low irradiance had plants that were sprayed and the control (unsprayed).

### 4.5.1 Growth

Sprayed plants in high irradiance gave higher leaf per day. This was significantly different between plants both sprayed and unsprayed in low LED irradiance, but not unsprayed plants in

high irradiance (Table 17). Low irradiance induced longer leaves in both sprayed and unsprayed plants compared with sprayed and unsprayed plants in high irradiance.

Unsprayed plants in low irradiance gave the least fresh weight compared with other treatments. Biomass accumulation was higher in sprayed plants in high irradiance compared with other treatments but not significantly different from unsprayed plants in high irradiance. There were no significant differences in water content between sprayed and unsprayed plants at either moderate or high irradiance, p=0.313 (Table17).

*Table 17. Effect of manganese (Mn) foliar spray (15ml/1L of water) on growth parameters of frillice lettuce growing in high (270 µmol $m^{-2}$ $s^{-1}$) and low (134 µmol $m^{-2}$ $s^{-1}$) irradiance with LED as the light source. The plants were grown at 20ºC day and 18ºC night temp with 70% RH for 17 days. The ANOVA results give the means, standard error (SE), and P-value of the treatments. Turkey test is used to compare the means of the treatments. Different letters in each column are significantly different at 5% level. In each treatment, N=7.*

| Treatment | Leaf per day | Longest leaf | Fresh weight | Dry weight | Water content |
|---|---|---|---|---|---|
| | | *cm* | *(g per plant)* | *(g per plant)* | *(%)* |
| LED-Mn (270 µmol $m^{-2}$ $s^{-1}$) | 1.03 AB ± 0.10 | 12.94 B ± 1.24 | 72.78 A ± 8.28 | 10.55 A ± 0.45 | 85.40 A± 1.13 |
| LED+Mn (270 µmol $m^{-2}$ $s^{-1}$) | 1.05 A ± 0.01 | 13.23 B ± 0.76 | 76.44 A ± 14.63 | 10.77 A ± 0.87 | 85.58 A ± 2.12 |
| LED-Mn (135 µmol $m^{-2}$ $s^{-1}$) | 0.92 C ± 0.03 | 15.26 A ± 0.57 | 56.19 B ± 3.21 | 8.76 B ± 0.18 | 84.37 A ± 0.68 |
| LED+Mn (135 µmol $m^{-2}$ $s^{-1}$) | 0.95 BC ± 0.04 | 14.91 A ± 1.30 | 58.34 B ± 4.85 | 8.88 B ± 0.21 | 84.71 A ± 0.97 |
| *P-value* | ***0.002*** | ***< 0.001*** | ***< 0.001*** | ***<0.001*** | ***0.313*** |

### 4.5.2 Tipburn assessment

Tipburn severity of the first 9 leaves of sampled plants (sprayed and unsprayed) from both moderate and high irradiance was almost the same (Figure 24). A difference in tipburn severity was observed from the 9$^{th}$ leaf where plants of moderate irradiance had reduced tipburn.

There was no significant difference in tipburn severity (both outer and inner) between sprayed and unsprayed plants at either moderate or high irradiance. However, high irradiance, both sprayed and unsprayed, induced more inner tipburn compared to plants of moderate irradiance (Figures 25a and 25b).

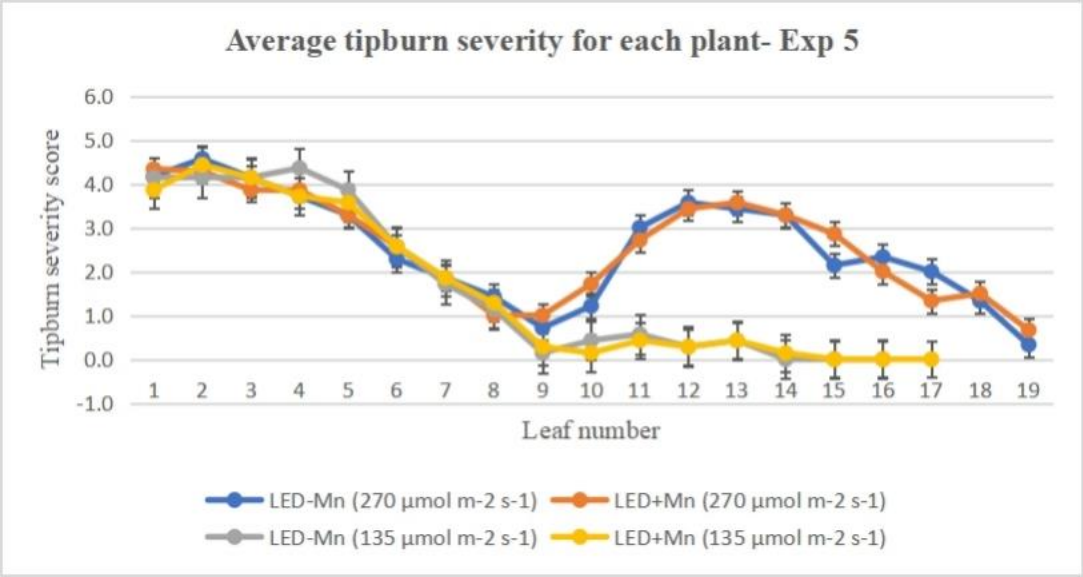



*Figure 24: Average tipburn score (0-5) (± SE) between plants grown with and without a foliar spray of Mn under high light with LED = LED+Mn (270 µmol $m^{-2}$ $s^{-1}$); LED-Mn (270 µmol $m^{-2}$ $s^{-1}$) and with and without a foliar spray of Mn under moderate light with LED= LED+Mn (135 µmol $m^{-2}$ $s^{-1}$); LED-Mn (135 µmol $m^{-2}$ $s^{-1}$) for each leaf for experiment 5. Leaf 1 is the first outer leaf after the cotyledon. A tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

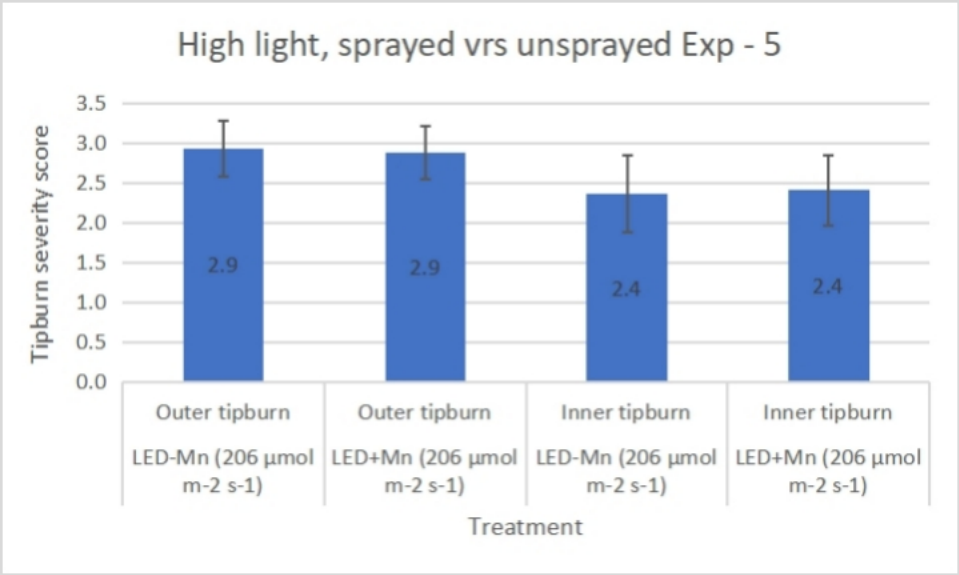



*Figure 25a: Average tipburn severity score (0-5) (± SE) between outer and inner leaves of plants grown with and without a foliar spray of Mn under high light with LED = LED+Mn (270 µmol $m^{-2}$ $s^{-1}$); LED-Mn (270 µmol $m^{-2}$ $s^{-1}$) for experiment 5. P-value for outer leaves is 0.949 and 1.000 for inner leaves. N= 7. Leaf 1 is the first outer leaf after the cotyledon. A tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

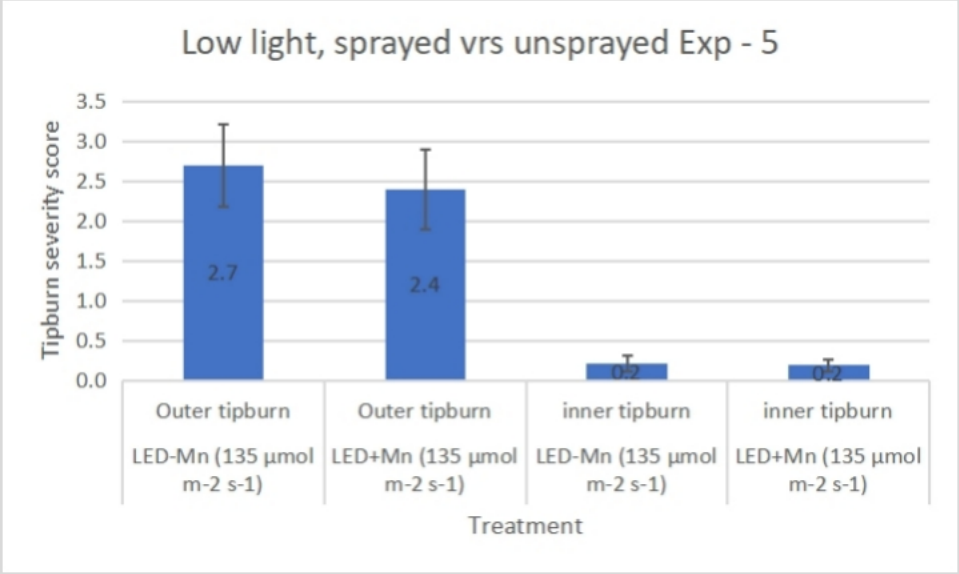



*Figure 25b: Average tipburn severity score (0-5) (± SE) between outer and inner leaves of plants grown with and without a foliar spray of Mn under moderate light with LED= LED+Mn (135 µmol $m^{-2}$ $s^{-1}$); LED-Mn (135 µmol $m^{-2}$*

*$s^{-1}$) for experiment 5. P-value for outer leaves is 0.180 and 0.198 for inner leaves. N= 7. Leaf 1 is the first outer leaf after the cotyledon. A tipburn score of 5 is the highest form of severity while 1 is the least severe. 0 is no tipburn.*

## 4.6 Antioxidant capacity (FRAP) results

FRAP analysis was performed on the inner leaves of plants from both treatments in experiment 3, low light (134 µmol m-2 s-1) with LED, and experiment 4, high light (206 µmol m-2 s-1) with LED to determine and compare their antioxidant capacity.

Plants from both treatments in high light (experiment 4) showed higher antioxidants in their inner leaves compared with plants from low light but the difference was not significant at 5% level (Table 18).

*Table 18: Effect of extra manganese in nutrient solution on plants grown under low irradiance (134 µmol m-2 s-1) compared with high irradiance (206 µmol m-2 s-1) on antioxidant capacity (FRAP) of Lactuca sativa L. under LED lamp.*

| Experiment | Treatment | LED+Mn | LED-Mn |
|---|---|---|---|
| 3 | (134 µmol $m^{-2}$ $s^{-1}$) | 2.04 ± 0.57 | 1.67 ± 0.50 |
| 4 | (206 µmol $m^{-2}$ $s^{-1}$) | 2.09 ± 0.86 | 2.80 ± 0.97 |
| | *P-value* | *0.917* | *0.050* |

### 4.6.1 Regression analysis

Finally, polynomial regression analysis was performed to find the correlation between tipburn of plants in high light and low light irrespective of HPS or LED used as light source and the amount of cations content in the leaves using Minitab (Table 19 and 20). The fitted line was plotted for manganese in both high and low light since the focus of the research work was on the addition of extra Mn in the nutrient solution.

There was a strong correlation between Mn content and the severity of the inner tipburn of plants in high light ($R^2$=63.2%). This was significant at 5% level (Table 19). Ca, Mg, and K explained 13.1%, Mg 26.6%, and K 5.0% of tipburn respectively (Table 19). The regression curve indicates that the risk for inner tipburn is low (below a tipburn score of 1) when the Mn content of the leaf is about 550 mg/kg – 1390 mg/kg (Figure 26).

In low light, 0.8%, 28.9%, 4.3%, and 26.8% of inner tipburn were explained by Ca Mg K, and Mn respectively (Table 20). Mn content between 0 and 1600mg/kg resulted in an inner tipburn score of less than 1 (Figure 27).

*Table 19: Coefficient of determination ($R^2$) predicting the incidence of inner tipburn by quadratic regression analysis at different cation content in the leaves of plants grown in high irradiance (206 µmol m-2 s-1) with either LED or HPS. N=25*

| | Ca | Mg | K | Mn |
|---|---|---|---|---|
| $R^2$ | 13.1% | 26.6% | 5.0% | 63.2% |
| value | 0.389 | 0.084 | 0.774 | <0.001 |

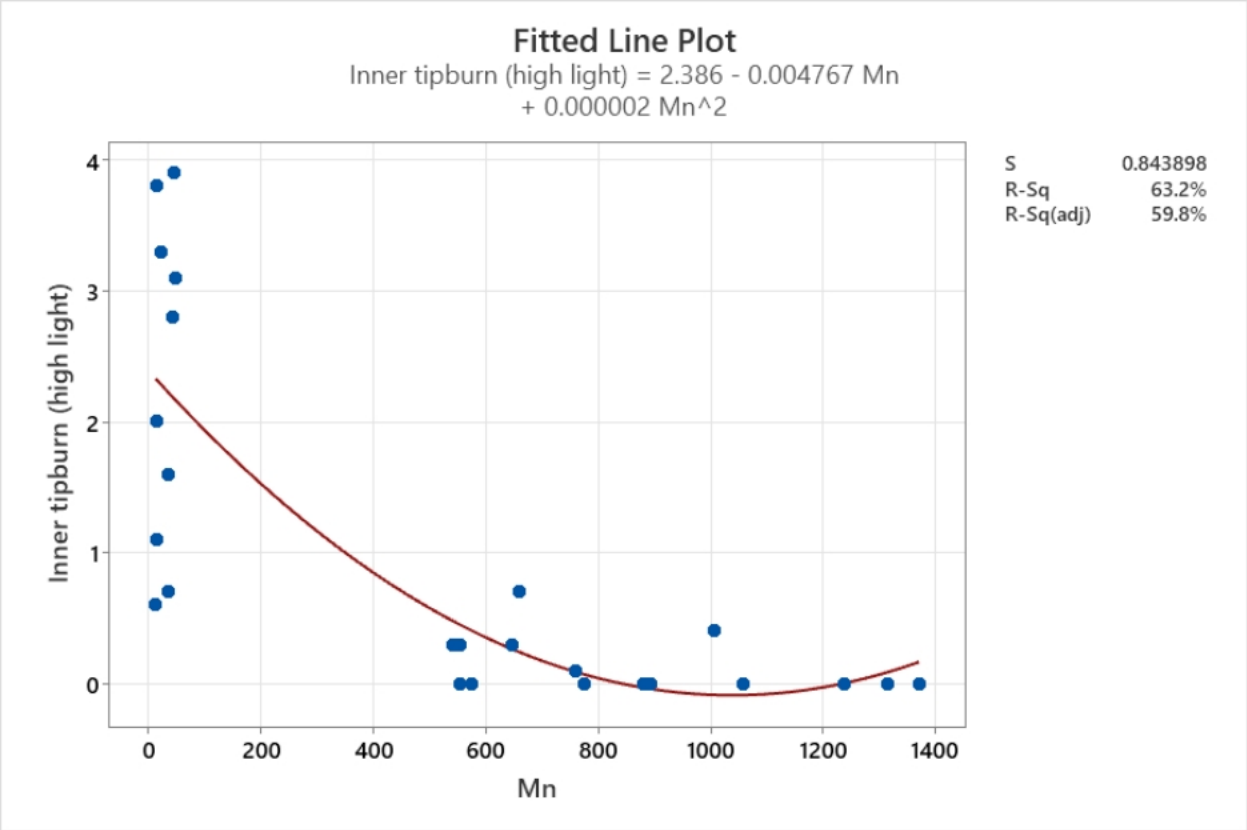



*Figure 26: Regression curve between inner tipburn severity and the amount of Mn accumulated in the leaves of plants grown under high irradiance in a growth chamber.*

*Table 20: Coefficient of determination ($R^2$) predicting the incidence of inner tipburn by quadratic regression analysis at different cation content in the leaves of plants grown in low irradiance (134 µmol m-2 s-1) with either LED or HPS. N=15*

| | Ca | Mg | K | Mn |
|---|---|---|---|---|
| $R^2$ | 0.8% | 28.9% | 4.3% | 26.8% |
| P-value | 0.993 | 0.270 | 0.767 | 0.311 |

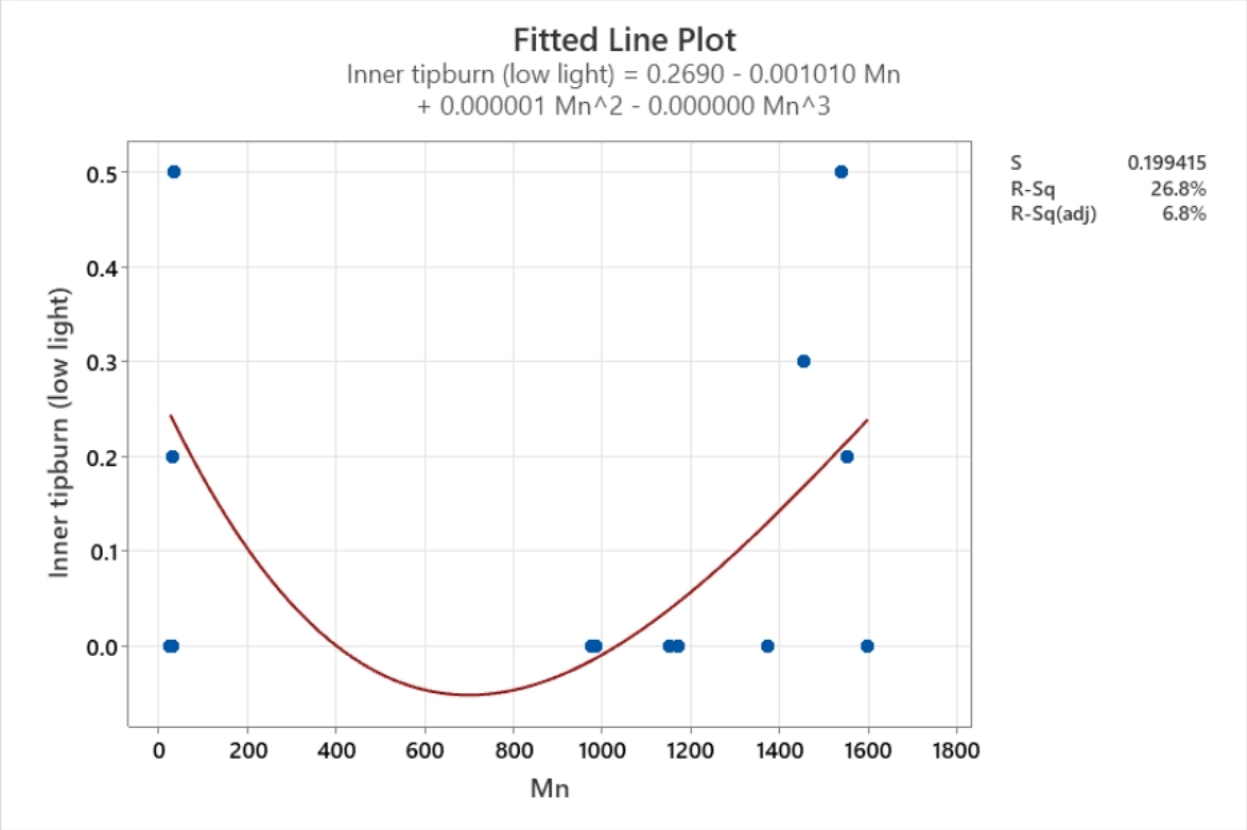



*Figure 27: Regression curve between inner tipburn severity and the amount of Mn accumulated in the leaves of plants grown under high irradiance in a growth chamber.*

# 5 Discussion

## 5.1 Nutrient accumulation

Although Mn is an essential micronutrient for plant growth and development, excess amounts can be toxic to plant cells (Alejandro et al., 2020). Increasing the concentration of manganese used in fertigation has been found to significantly influence the content of Mn in plants (Kleiber, 2014). Also, too high concentrations in the growing medium often led to competitive

interference with other elements like Fe, Mg, Ca, and K (St. Clair & Lynch 2004). The intensity of manganese toxicity increases when the quantity of other available elements like Ca, Mg, K, Fe, and Si are low (Millaleo et al., 2010).

According to Bævre & Gislerød (1990), Mn content of <20, 50-200, and >200 µg/g dry matter in the leaves of lettuce is defined as a deficiency, normal, and toxic respectively (Table 21). In this study, the leaves were divided into different parts to better understand how Mn accumulates at different parts of the plant. Mn content was different at various parts of the leaf both in the control and treated plant. Higher Mn content was found in all parts of leaves treated with an extra 111ppm of Mn in the nutrient solution (Table 21). This agrees with the findings of Przybysz et al. (2017), who found higher Mn content in leaves of lettuce plants treated with extra Mn. With reference to the work of Bævre & Gislerød (1990), the levels of Mn in treated leaves were toxic in all parts analyzed (Mn > 665.4) (Table 21) whilst the control had either deficiency or close to deficiency in young and inner parts of old leaves. The highest contents of Mn were found in the edges of old leaves treated with extra Mn. Movement of Mn from the roots into the xylem to photosynthetically active leaves through the transpiration stream have been reported (Millaleo et al., 2010). About 90% of total transpiration in leaves is stomatal (Marschner, 1995). Old leaves have a lot of stomata and transpire more and therefore have a high accumulation of elements in that part. This might explain why higher Mn contents are found in the edges of old leaves which was the case in this study. Mn is less mobile in the phloem. This leads to a lower accumulation of Mn in young leaves. The effect of light was dependent on lamp type (HPS and LED). Higher content of Mn was found in leaves exposed to HPS compared with LED. The rate of transpiration and potential uptake and translocation of elements are higher during light periods (Marschner, 1995). This was seen in the work of McCain & Markley (1989), who found higher Mn content in sun leaves than in shade leaves of maple trees of the same leaf age. In this study, plants in HPS had 18 hours of photoperiod whilst plants in LED had 16 hours of photoperiod. This might also explain why there was higher Mn content in treated leaves under HPS. HPS also has more infra-red radiation, and this can increase transpiration. It is possible that lettuce produced with HPS has higher transpiration compared to LED, but this was not measured.

Antagonistic effect of increased Mn on elements like Ca, Mg, and K have been reported. This was confirmed in the work of Lee et al., (2011) who found decreased contents of Ca K, Mg, and

Zn in the outer leaves of Chinese cabbage with elevated Mn concentration. (Kazda & Zvacek, 1989) also found a 50% reduction in uptake of Ca and Mg when additional manganese of 5.5 mg. $L^{-1}$ was added to the growing medium. In this study, extra Mn had little effect on Ca, Mg and K when HPS was used as a light source which was different at different parts of the leaf. Under LED lamp, there was no significant effect on Ca and K, but the content of Mg was significantly reduced when extra Mn was added to the nutrient solution. Zn in most cases was below detection level. The content of the cations was higher in the edge of older leaves just as Mn except for K which accumulated in the inner part of old leaves irrespective of lamp type and irradiance. Different antagonistic effects might be explained by the difference in Mn concentration, irradiance, part of the plant analyzed, and lamp type.

*Table 21: Summary of Manganese content (mg/kg) in parts of fillice lettuce leaves treated with extra Mn in nutrient solution = +Mn, and without extra Mn in nutrient solution = -Mn. from experiments 1, 2, 3, and 4 (see results section) and reference values from Bævre & Gislerød (1990), HPS/HI= High pressure sodium with high irradiance (300 µmol $m^{-2}$ $s^{-1}$), HPS/MI=High pressure sodium with moderate irradiance (150 µmol $m^{-2}$ $s^{-1}$), LED/LI= Light emitting diode with low irradiance (134 µmol $m^{-2}$ $s^{-1}$) and LED/HI= Light emitting diode with high irradiance (206 µmol $m^{-2}$ $s^{-1}$).*

| Treatment | Young leaves | | Edge of old leaf | | Inner part of old leaf | | Reference (µg/g) (Bævre & Gislerød, 1990) | | |
|---|---|---|---|---|---|---|---|---|---|
| | +Mn | -Mn | +Mn | -Mn | +Mn | -Mn | Deficiency | normal | toxic |
| HPS/HI | 665.4 | 14.95 | 4421 | 194.7 | 2072 | 34.27 | < 20 | 50-200 | > 200 |
| HPS/MI | 1504.2 | | 8163 | | 2230.5 | | | | |
| LED/LI | 1054.3 | 30.54 | 3706 | 168.36 | 1830.3 | 40.71 | | | |
| LED/HI | 888 | 41.05 | 4096 | 350.2 | 2136 | 124.9 | | | |

## 5.2 Extra manganese and biomass accumulation

Lettuce plants treated with elevated Mn will have lower photosynthesis leading to less biomass accumulation (Przybysz et al., 2017). The reduction of photosynthetic intensity was attributed to reduced stomatal conductance, making $CO_2$ flow to the chloroplast more difficult. However, the decline in intensity depended on the cultivar. In the work of Shi et al. (2006), excess Mn with optimum light intensity enhanced oxidative stress in Cucumis sativus plants following a reduction in growth.

In this study, plant biomass was not affected by Mn in HPS irrespective of irradiance. However, leaf length was affected depending on the period the treatment was given. Longer leaves were recorded when the Mn was given in the last two weeks of the plant growth. Under LED lamp, biomass decreased with elevated Mn either in low light or high light. However, longer leaves were recorded in low light with extra Mn. Lee et al. (2011), found a reduction in leaf length, leaf size, and fresh and dry weight of shoots of Chinese cabbage with 1.5 mM Mn treatment. Przybysz et al. (2017), also found a reduction in the biomass accumulation following a reduction in the efficiency of the photosynthetic apparatus in lettuce treated with Mn. However, other reports have shown that the inhibition of photosynthesis in Mn-treated plants is a result of severe chlorosis affecting stomatal conductance. Impairment of photosynthetic apparatus in elevated Mn might also lead to a reduction in the number of chloroplasts and chlorophyll content (Demirevska-Kepova et al., 2004). All this affects plants' growth. However, in the present study, no chlorosis or visible symptoms of chlorophyll reductions were found in any of the experiments. Hence, the effect of Mn on growth is depending on the concentration. Too high Mn content will eventually lead to growth reduction. In the present study, the Mn content was not high enough to strongly restrict the growth.

The differences seen in growth pattern with extra Mn in HPS and LED implies that light spectra have a role in plants' photosynthetic efficiency under elevated Mn. Light harvesting complexes of plants (chlorophyll a and chlorophyll b) have peak absorptions in red and blue light. Red light affects photosynthetic apparatus development whilst blue light influences stomatal opening (Paradiso & Proietti, 2021). These two lights particularly influence plant photosynthesis (Ahmed et al., 2020) where excess light can cause photodamage. The spectral distribution of LED used in

this work has more red and blue light (see figure 11). The combined effect of high irradiance with these spectra in addition to excess Mn might have resulted in more oxidative stress (more ROS) and caused damage to the photosynthetic apparatus resulting in higher tipburn severity and a reduction in plant growth. Irradiance below 134 µmol $m^{-2}$ $s^{-1}$ is recommended when using LED with more red and blue light to help reduce tipburn and increase biomass.

In the work of Hytönen et al., (2017), LED and HPS showed no difference in the yield of Frillice´ lettuce in greenhouse production. This seems to be different when nutrient solution is manipulated. Another explanation based on this study could be the competition between Mn and other elements required for photosynthesis. High Mn content in leaves inhibits RuBP carboxylase reaction thereby decreasing net photosynthesis (Marschner, 2011). Clair & Lynch (2004) found decreased Mg and K when Mn was high, both of which are involved in photosynthetic electron transport, the activity of RuBisCO, and regulation of the stomata.

In this study, Mg in most cases was significantly reduced. Reduced concentration of this element might have led to reduced photosynthesis affecting the plants' growth under the increased concentration of Mn under the LED lamp. Under HPS, Mg content was not affected. The increased Mn might have helped the plants to produce more antioxidants and antioxidative enzymes like the Mn-SOD to cope with ROS produced in high light and high Mn concentration to help the plants continue with growth. This was evident in the Mn content of treated leaves under HPS which also corresponded with reduced average outer tipburn than treated plants in LED. On the other hand, foliar spray with Mn did not have any effect on plant growth and biomass.

## 5.3 Light and extra Mn on tipburn severity

Excess of Mn reaching toxicity levels can be detrimental to plant cells. Toxicity alters physiological, biochemical, and molecular processes at the cell level. There is also alteration of photosynthetic apparatus and the photosynthetic performance of plants by Mn toxicity. Toxicity symptoms include chlorosis, necrosis of leaves, and browning of roots. In the work of Lynch & Clair (2004), Mn toxicity triggered oxidative stress by generating OH (ROS) which is harmful in

plant cells. The response of plants to excess Mn is affected by many factors including light intensity (González et al., 1998). Different reports have shown that crops grown in high light get more serious symptoms of Mn toxicity than those grown in low light (González et al., 1998).

Panda et al, 1986 showed lipid peroxidation in isolated chloroplast of aging wheat with excess Mn. Knoop (2019) also found more inner tipburn in frillice lettuce under high light intensity irrespective of the light quality. Przybysz et al. (2017), have suggested that excess $Mn^{2+}$ induce oxidative stress which in turn, activates antioxidative enzymes including the Mn-SOD. However, in experiment 1-4, although Mn contents in the inner leaves of treated plants were toxic with reference to Bævre & Gislerød (1990), there was a good correlation between inner leaf tipburn and excess Mn in nutrient solution irrespective of irradiance (Figure 26 and 27). The extra Mn in the nutrient solution might have helped alleviate toxicity with an increase in antioxidants. In the control plants, lower content of Mn (14.95 - 41.05mg/kg) in the inner leaves resulted in higher tipburn severity except in low light with LED where the average tipburn severity score was less than 1. With reference to Mn content in lettuce reported by Bævre & Gislerød (1990), tipburn in the inner leaves of control plants may be a result of deficiencies. However, low irradiance may reduce the effect of deficiency as seen in plants in low light with LED. Mn content in the leaf edge and outer tipburn did not correlate. The effect on outer leaf tipburn was dependent on lamp type. For example, in this study, plants treated with extra Mn in the nutrient solution and accumulating 8163mg/kg in the edge of old leaves resulted in an average tipburn of 1.6 in 150 µmol $m^{-2}$ $s^{-1}$ with HPS whilst treated plants in 206 µmol $m^{-2}$ $s^{-1}$ with LED accumulating 4096mg/kg in the edge of old leaves resulted in an average outer tipburn of 2.6. This implies the importance of light spectra might also play a role in tipburn when the nutrient solution is manipulated. More red and blue light in LED used in this experiment in combination with excess Mn might have led to the production of reactive species (oxidative stress). This effect was seen in outer tipburn severity of plants grown with LED compared with plants grown with HPS (Figure 17b,18b, 20b and 23b).

Foliar application of extra Mn of 15ml $L^{-1}$ did not give any difference between treated plants and untreated plants either in high irradiance or low irradiance. However, more tipburn (both inner and outer leaves tipburn) was recorded in plants in high light. The result agrees with González et al. (1998), who found more chlorosis in leaves exposed to high light than in plants receiving less

light at similar foliar Mn content. It also possible that higher concentration and more frequent spraying would have an effect. The lettuces were only sprayed 3 times.

## 5.4 Extra Mn and antioxidant capacity

Inner leaf tipburn is of so much importance to frillice lettuce growers since it can lead to rejection of the whole plant and can also serve as a gateway to fungi and bacteria infections. A reduction in inner leaf tipburn, especially in low irradiance with LED, recording zero severity score with elevated Mn. Therefore, it was of interest to investigate the antioxidant capacity of the plants to find out if there was a link between antioxidant capacity, Mn content, and tipburn severity.

Antioxidants are involved in scavenging excess ROS which increases in plants exposed to high irradiance (light stress) and/or treated with excess Mn (Przybysz et al., 2017). Li et al. (2010), claim that the antioxidant systems in plants treated with excess Mn provide enough protection against oxidative damage in such plants. In the work of Shi et al., (2006) antioxidants like superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) increase with excess Mn under optimum light intensity in cucumber. The results showed an increased antioxidant capacity of treated plants in high light compared with treated plants in low light. However, between treatments with extra Mn and without extra Mn, high antioxidant capacity was found in the treatment without extra Mn in high light whilst the opposite was seen in low light. The reduction of antioxidant capacity in treated plants may be attributed to depleted antioxidants in response to increased ROS production from increased light and excess Mn.

It has also been shown in the work of Przybysz et al. (2017), that increased activity of ascorbate peroxidase (APX), catalase (CAT), and a higher content of phenols in leaves of lettuce treated with extra Mn corresponded with increased ROS formation in aerial parts of the plants.

Although inner tipburn was not completely combated under high light, the increased antioxidant capacity of the plants might be because of increased ROS produced from the combined effect of

high light and excess Mn which might not be the case in low light. The involvement of antioxidative enzymes in scavenging ROS under excess Mn is reported by Srivastawa & Dubey, (2011). Oxidative stress under Mn toxicity might be a result of depletion of antioxidants as they are involved in the detoxification processes of scavenging ROS. Since the activity of individual antioxidative enzymes were not measured, there could also be a misbalance among antioxidative enzymes whose sensitivity to extra Mn varies, i.e. The activity of SOD requires Mn, but CAT and APX do not.

## 5.5 The practical implications for growers

Farmers have long been looking for solutions to combat tipburn to avoid losses. The cause of tipburn has been attributed to many external factors including climate and nutrients. This makes it more difficult to deal with the problem since the greenhouse climate can vary widely and some of them can be difficult to control. Researchers have suggested different solutions to help reduce tipburn in lettuce. Knoop (2019), recommended the use of supplemental lighting less than 17 mol/day. Lee et al. (2013), reduced tipburn in lettuce with 24-hour airflow above 0.28m.s-1 along cultivational beds. Vanhassel et al. (2014), also showed 95% night air humidity to reduce tipburn by 50% in butterhead lettuce.

Mn is an essential micronutrient that affects both plant products and their consumers. In humans, manganese is important for various metabolisms and is an antioxidant aiding in defense against free radicals. However, intake beyond recommended levels can cause neurotoxicity leading to health implications (Aschner & Aschner, 2005). Vegetable like lettuce is a dietary source of Mn in the human diet. In plants, Mn content varies between 15-100 µg g-1 dry weight (Asati et al., 2016). Average consumption of 3–5 mg per day is recommended for adults (varying between men and women) and 0.5 - 2 mg for children depending on age (Sidorova et al., 2020). From this thesis, the use of extra Mn of 111 ppm in the nutrient solution will help growers to reduce inner leaf tipburn. However, the concentration of Mn in the leaves will be too high for human consumption

Further research using less Mn than 111ppm is recommended. The distribution of Mn revealed in this work can be good information for breeders to develop frillice lettuce that better distributes Mn in their tissues to reduce the tipburn problem.

# 6 Conclusions

- The Mn content of inner leaves is much lower than the edge of outer old leaves and follow a similar pattern of distribution as Ca.
- Increased Mn content in the nutrient solution led to a higher concentration of this element in all parts of frillice lettuce analyzed.
- Foliar application of extra Mn did not affect plant growth nor tipburn severity (both inner tipburn and outer tipburn) either in low light or high light with LED.
- Increased Mn content in the inner leaves led to less inner tipburn.
- The effect of extra Mn in the nutrient solution on outer tipburn severity was dependent on lamp type and irradiance
    - Severity was high in plants grown in high irradiance with LED lamp.
- The effect of extra Mn in the nutrient solution on plant growth was dependent on lamp type and irradiance
    - Low light with LED did not significantly affect fresh weight whilst high light with LED significantly reduced it.
    - Fresh weight and dry weight were significantly increased in high light with HPS.
- Extra Mn in the nutrient solution had no effect on the accumulation of Ca, K and Mg under HPS but significantly reduced Mg were found under LED.
- Antioxidant capacity was high in plants exposed to LED in high light but did not change in response to Mn content and was not correlated with tipburn incidence.
- Additional Mn can reduce inner tipburn in lettuce, but levels will be too high for human consumption.

# 7 Reference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# 8 Appendix 1- NLR registration form for outer and inner tipburn

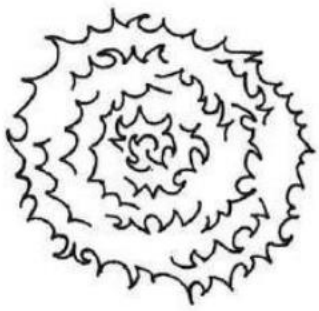
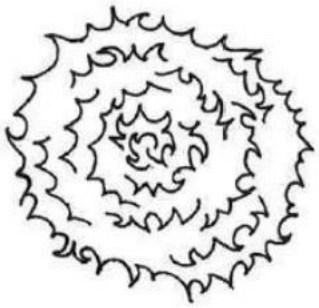
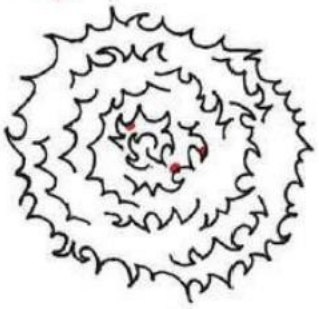
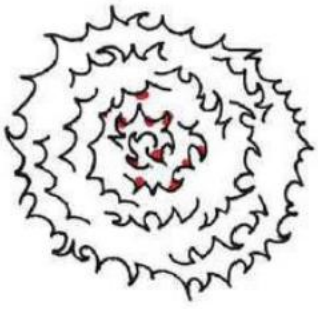
| Skala | Forklaring | Skisse ytre bladrand | Skisse indre bladrand |
|---|---|---|---|
| 0 | Ingen synlig bladrandskade |  |  |
| 1 | Små brune flekker i enkelt bladspisser |  |  |
| 2 | De fleste bladspisser er brune i spissen |  |  |

*Figure 1: Norwegian Extension Service (NLR) registration form.*

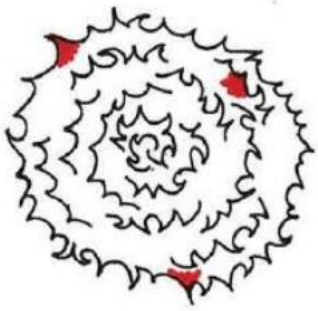
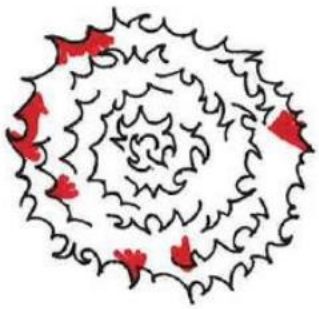
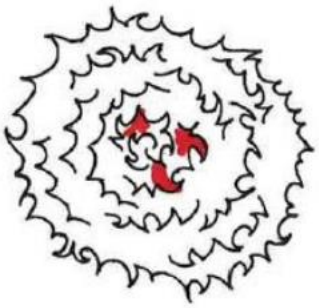
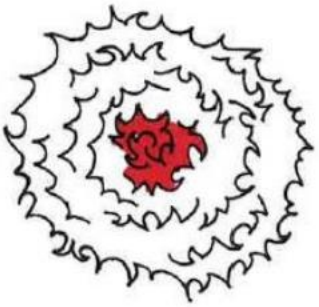
| | | | |
|---|---|---|---|
| 3 | Enkelte til flere bladspisser helt brune/visne |  |  |
| 4 | Større deler av enkelte blader påvirket av bladrandskade |  |  |
| 5 | Større dele av de fleste blader påvirket av bladrandskade |  |  |

*Figure 2: Norwegian Extension Service (NLR) registration form.*

## Appendix 2- images of some inner and outer tipburn assessment.

*Figure 3: No inner leaves tipburn of a plant from experiment 3 treated with extra Mn in the nutrient solution. Photo: Sissel Torre*

*Figure 4: inner leaves tipburn of plant treated with nutrient solution without extra Mn.*

*Figure 5: Entire plant tipburn showing both inner (black circle) and outer (blue circle) leaves.*

*Figure 6: Outer tipburn*

*Figure 7: inner tipburn*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| **Student(s):** | |
|---|---|
| Student(s)' name: | Gifty Kodua |
| Student number: | 106273 |
| Study program: | Plant Science |

| Supervisor: | |
|---|---|
| Main supervisor: | Sissel Torre |
| Co-supervisor/ external supervisor: | |

| Degree thesis: | |
|---|---|
| **NB:** NMBU uses the title of the degree thesis on transcripts and diplomas, it is recommended that the title of the thesis does not contain any confidential information. | |
| Thesis title: | **The role of manganese in light stress-induced tipburn and growth of lettuce *(Lactuca sativa L."Frillice")*** |
| The thesis will be submitted at faculty: | Faculty of Biosciences |

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| Signatures: *Must be completed | | |
|---|---|---|
| | Dato: | Signature: |
| Student(s)* | 05.05.2022 | |
| Main supervisor* | 05.05.2022 | Sissel Torre |
| Co-supervisor/ external supervisor | | |

| | | |
|---|---|---|
| Dean or the person the dean has authorized* | 11.05.2022 |  |
| Institution/company | | |