Assessing the effect of planting density on romaine lettuce growth and quality in a controlled hydroponic environment

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A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science.

August 2023

Abstract

Efficient utilization of planting areas in controlled environment agriculture (CEA) is essential for maximizing crop yield. This thesis focuses on the optimization of plant spacing and planting patterns for romaine lettuce (*Lactuca sativa*) in a controlled environment hydroponic system. By identifying the optimum planting area, resource use efficiency and profit of CEA facilities can be improved.

The first study aimed to determine the optimum plant spacing for romaine lettuce in terms of yield and head development. Different planting densities and patterns were tested, ranging from 388 (5.0 cm) to 32 (17.7 cm) plants per square meter. Staggered and non-staggered planting patterns were employed in this study to investigate their effect on plant growth and development. Fresh mass, dry mass, plant height, internodal distance, head and heart diameter head and heart leaves were taken into consideration in this experiment. The heart refers to the central portion of the lettuce head where the leaves are compact and tightly packed, providing a firm structure to the lettuce. The results demonstrated that lower spacing conditions promoted bolting and internodal elongation leading to poor yield. Increasing the plant spacing significantly increased the fresh and dry mass of romaine lettuce, reaching a yield plateau for dry mass at a spacing of 17.2 cm. Fresh mass yield plateau started at 20 cm plant spacing and plant spacing beyond 25 cm was found to be non-profitable indicating that Valley Heart has attained its yield plateau around this spacing. Staggered planting patterns had no significant impact on head and heart development. Head and heart formation were only observed within the planting spacing range of 15.5 cm to 17.7 cm. The study revealed the negative effects of internodal elongation and plant lodging in high density planting conditions.

The second study focused on plant competition and compared the responses of two romaine lettuce cultivars, 'Valley Heart' and 'Breen', to different planting densities. Four spacing conditions (6.3 cm, 7.6 cm, 8.8 cm, 10.1 cm) with both staggered and non-staggered planting patterns were implemented in an ebb-and-flow hydroponic system. The results showed that Valley Heart had a higher yield at lower spacing conditions, while Breen demonstrated the potential to form heads and hearts at lower-density conditions. However, Valley Heart tended to bolt under these lower-density conditions, affecting its overall productivity. While head and heart development were observed in Breen in 8.8 cm and 10.1 cm spacing configurations. These findings contribute to a

better understanding of how different cultivars respond to planting density, offering valuable insights for optimizing lettuce cultivation in hydroponic systems.

Overall, these studies emphasize the significance of planting density and patterns in CEA. By selecting appropriate plant spacing, planting patterns, and suitable cultivars, growers can enhance yield, head development, and resource use efficiency while minimizing internodal elongation and plant lodging issues. Understanding the responses of different cultivars to planting density allows for more informed decision-making in controlled environment agriculture, ultimately improving crop productivity and profitability.

Résumé

L'utilisation efficace des surfaces de plantation en agriculture en environnement contrôlé (AEC) est essentielle pour maximiser le rendement des cultures. Cette thèse se concentre sur l'optimisation de l'espacement des plants et des motifs de plantation pour la laitue romaine (*Lactuca sativa*) dans un système hydroponique en environnement contrôlé. En identifiant la zone de plantation optimale, l'efficacité de l'utilisation des ressources et le profit des installations d'AEC peuvent être améliorés.

La première étude visait à déterminer l'espacement optimal des plants de laitue romaine en termes de rendement et de développement de la tête. Différentes densités de plantation et motifs ont été testés, allant de 388 (5.0 cm) à 32 (17.7 cm) plants par mètre carré. Des motifs de plantation échelonnés et non échelonnés ont été utilisés dans cette étude pour étudier leur effet sur la croissance et le développement des plantes. La masse fraîche et sèche, la hauteur des plants, la distance internodale, le diamètre des têtes et des feuilles ont été pris en compte dans cette expérience. Le groupe compact et densément emballé de feuilles, offrant une structure solide à la laitue, est communément appelé "tête". Le "coeur" désigne la partie centrale de la "Valley Heart" d'où proviennent ces feuilles. Les résultats ont montré que des conditions d'espacement plus réduites favorisaient le montaison et l'allongement des entrenœuds, entraînant ainsi un faible rendement. En augmentant l'espacement entre les plants, la masse fraîche et sèche de la laitue romaine a significativement augmenté, atteignant un plateau de rendement pour la masse sèche à un espacement de 17.7 cm. Le plateau de rendement en masse fraîche a commencé à un espacement de 20 cm, et un espacement supérieur à 25 cm s'est avéré non rentable, ce qui indique que Valley Heart atteint son plateau de rendement autour de cet espacement. Les schémas de plantation en quinconce n'ont eu aucun impact significatif sur le développement des têtes. Le développement des têtes n'a été observé que dans une plage d'espacement de plantation de 15.5 cm à 17.7 cm. L'étude a également révélé les effets négatifs de l'élongation des entre-nœuds et du couchage des plants dans des conditions de plantation à haute densité.

La deuxième étude s'est concentrée sur la compétition entre les plantes et a comparé les réponses de deux cultivars de laitue romaine, 'Valley Heart' et 'Breen', à différentes densités de plantation. Quatre conditions d'espacement (6.3 cm, 7.6 cm, 8.8 cm, 10.1 cm) avec des motifs de plantation échelonnés et non échelonnés ont été mises en œuvre dans un système hydroponique à

flux et reflux. Les résultats ont montré que 'Valley Heart' avait un rendement plus élevé dans des conditions d'espacement réduit, tandis que 'Breen' avait la capacité de former des têtes et des cœurs dans des conditions de densité plus faible. Cependant, 'Valley Heart' avait tendance à monter en graines dans ces conditions de densité plus faible, ce qui affectait sa productivité globale.

Ces études mettent en évidence l'importance de la densité de plantation et des motifs de plantation en AEC. En choisissant un espacement adéquat entre les plants, des motifs de plantation appropriés et des cultivars adaptés, les producteurs peuvent améliorer le rendement, le développement de la tête et l'efficacité de l'utilisation des ressources, tout en minimisant les problèmes d'allongement des entrenœuds et d'affaissement des plantes. Comprendre les réactions des différents cultivars à la densité de plantation permet de prendre des décisions éclairées en agriculture en environnement contrôlé, ce qui améliore finalement la productivité et la rentabilité des cultures.

Acknowledgments

First, I would like to thank my supervisor, Dr. Mark Lefsrud, who allowed me to demonstrate my abilities. I am grateful for his continuous support, impulse, guidance, scholastic advice, and invaluable recommendations for the research. Thanks for giving me the liberty in research and unwavering time to carry out this study. Also, I will follow the lab safety and be on time in the future per your advice. His endurance, financial and moral support is greatly acknowledged. If, by some cosmic coincidence, a rare typo or error manages to sneak in, I have no doubt it's just a tribute to Dr. Lefsrud' s extraordinary ability to encourage creative interpretation. Gratitude is expressed to Rvest (Up culture) for the financial support of this project. I would also like to thank Dr. Shangpeng Sun, who mentored me in my research. His diligent guidance and counsel were of great help during my master's.

I am forever grateful to my beloved friend Naresh Kumar Arumugagounder Thangaraju, who acted as my guardian in Canada. His valuable teachings, guidance, and moral, and academic support were a great help to me in finishing up my thesis. His presence made me come out of homesickness and helped me brainstorm research ideas. If I had the option to share this master's degree, I would be doing that with my dear friend Olivia Mendelson who has constantly supported me academically and emotionally and contributed immensely to this research throughout my master's journey. Her invaluable assistance in this project made this thesis possible. I am equally thankful to Dr. Sarah MacPherson for her constant encouragement, academic and emotional support, and steadfast presence throughout my master's journey. I am deeply appreciative of her exceptional support, especially in reviewing my manuscripts relentlessly, which I value tremendously.

I am grateful to Sai Uday Reddy Sagili for his unwavering presence, continuous moral support, and invaluable encouragement and help during my challenging times. I sincerely appreciate Jérôme Trudel-Brais, who helped me build the data collection environmental system. I would like to thank my other lab mates Philip, Sophie, Shafieh, Nastran, and Felix who helped me throughout my master's journey. I would also like to thank Sarah-Ann and Michael from greenhouse for their invaluable help and support during challenging times.

I would like to extend my heartfelt thanks to Dr. Arivudai Nambi Sundararajan, Dr. Senthil Kumar Palanivel, Dr. Sivakumar, Dr. John Christy Robert, and Dr. Ravikumar for their invaluable encouragement and the commendable recommendation letters they provided which helped me to attain my master's in McGill. I would like to thank my friends Harini, Smriti, Sujatha, Vijay Kamesh, and Keerthana who have supported me emotionally throughout this incredible journey. Special thanks to Ragul Nivash for their invaluable support and presence during crucial moments.

I am sincerely grateful to my best friend, Kareem Basha, for his unconditional support throughout this journey. I am grateful for his constant encouragement and comfort, standing by me through the highs and lows, including my emotional breakdowns.

Last but not least, I must thank my parents, Antony Samy, and Innocentia Selva Mary, for their sacrifices, love, and prayers. I am grateful to them for supporting me emotionally and financially throughout this journey and thankful for always trusting me. I would also love to express my heartfelt appreciation for my sister, Ancy Liyana, who has been an immense source of motivation and emotional support throughout this journey.

Contribution of authors

In adherence with the McGill Guidelines for a Manuscript-Based Thesis, this section outlines the contributions made by the candidate and co-authors in completing this work. Eugene Roy Antony Samy is the principal author of all the chapters in this thesis, supervised by Dr. Mark Lefsrud from the Department of Bioresource Engineering, McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada.

Dr. Mark Lefsrud, the supervisor, co-authored all manuscripts and offered scientific guidance throughout the research process, including designing and implementing the research work, co-editing, and reviewing the manuscripts.

Olivia Mendelson aided in the experiments, helped in the preparation of original drafts, and reviewed and co-authored all manuscripts.

Dr. Sarah MacPherson reviewed and improved all the manuscripts and co-authored all manuscripts.

Naresh Kumar Arumugagounder Thangaraju helped with the statistical design of this experiment and the data analysis of the manuscript.

Jérôme Trudel-Brais aided the experiment by building raspberry pi for environmental data collection.

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List of Abbreviations and Acronyms

ABA – Abscisic acid

ANOVA – Analysis of variance

BC – Before Christ

Ca – Calcium

CAD – Canadian dollars

CEA – Controlled environment agriculture

CO_2 – Carbon dioxide

DLI – Day light intensity

DO – Dissolved oxygen

DWC – Deep water culture

E. coli - Escherichia coli

EC – Electrical conductivity

FAO – Food and Agriculture Organization

Fe – Iron

FL – Fluorescent lights

h – Hours

H_2O – Water

HAF – Horizontal air flow

HPS – High-pressure sodium

INC – Incandescent

INSV – Impatiens necrotic spot virus

IPAR – Interception of photosynthetically active radiation

LED – Light emitting diode

LSD – Least significant difference

Mg – Magnesium

mL – Milli liters

Mn – Manganese

NFT – Nutrient film technique

Nm – Nanometer

NOSB – National organic standards board

PAR – Photosynthetic active radiation

PFEL – Plant factories with electrical lighting

pH – Potential hydrogen

PPD – Planting population density

PPFD – Photosynthetic photon flux density

PPO - Poly phenol oxidase

RH – Relative humidity

SD – Standard deviation

TDS – Total dissolved solids

VPD – Vapor pressure deficit

Chapter 1

General introduction

This chapter presents the background and rationale for the development of the research project, along with a statement of the research problems and objectives of this study.

1.1 Thesis motivation

Lettuce (*Lactuca sativa*) is a popular leafy green vegetable widely consumed for its crisp texture, mild flavor, and high nutritional value. It is cultivated worldwide in various climates and conditions. Lettuce is a suitable crop for large-scale commercial production since it is in great demand and has a high value in urban and peri-urban areas. Lettuce contains a significant amount of edible biomass as well as several essential minerals, vitamins, and fiber (Weber, 2016). As adequate nutrient concentrations can be given in the water, lettuce is an essential vegetable grown under hydroponics. To achieve the best results in lettuce production, farmers and agronomists need to carefully consider the planting density, which involves finding the right balance between plant spacing and population density.

Planting density is a critical factor in agricultural practices that influences yield, crop growth and development. It refers to the spacing arrangement of plants within a given area and plays a significant role in optimizing resource utilization, maximizing production efficiency, and achieving desirable crop outcomes. In soil-based agriculture, crop density is determined based on various factors, including soil fertility, water availability, sunlight exposure, and the specific requirements of the crop being grown. In contrast, hydroponic systems grow plants without soil, and their roots are immersed in a nutrient-rich water solution. In hydroponics, crop spacing density can be more precisely managed due to the controlled environment. The spacing is adjusted based on factors such as the specific hydroponic setup, the crop type, and the desired growth characteristics. Moreover, maximizing lettuce yield in CEA is more critical due to the higher cost of CEA structures and energy usage. Increasing yield is essential to improve the return on investment and make CEA economically viable. When it comes to lettuce cultivation, determining the appropriate planting density is a crucial factor to ensure optimal plant growth, yield, and overall crop performance. The choice of planting density can significantly affect lettuce plants' access to light, water, and nutrients, as well as their ability to compete with neighboring plants. Insufficient spacing may lead to overcrowding, resulting in poor air circulation, increased disease susceptibility, and reduced individual plant growth. Excessive spacing can result in space wastage compromising overall yield potential. The optimal planting density for lettuce depends on several factors, including the lettuce variety, environmental conditions, available resources. Different lettuce cultivars exhibit varying growth habits, leaf sizes, and physiological characteristics, which can influence their response to planting density. Environmental factors such as temperature, humidity, and light availability impact the optimal spacing requirements for lettuce. Finding the right planting density is a delicate balance between maximizing land use efficiency and ensuring individual plant performance. By optimizing planting density, growers can achieve uniform plant development, enhance light penetration, promote air circulation, and improve overall crop health. Moreover, an appropriate planting density can lead to increased yield potential, improved harvest efficiency, and enhanced crop quality.

In conclusion, by determining the right balance between plant spacing and population density, growers can maximize resource utilization, improve crop health, and achieve optimal yield and quality outcomes in lettuce production.

1.2 Research problem

Optimizing the planting density can improve the resource use efficiency and maximize the yield in a controlled environment hydroponic system.

1. Investigate the effect of planting density and planting pattern on romaine lettuce yield and morphological characteristics in a controlled environment hydroponic system.
2. Determine the ideal spacing to reduce the plant height and internodal elongation thereby head and heart formation can be improved in the romaine lettuce cultivars.
3. Determine factors for an ideal romaine lettuce cultivar which can form a head and heart in high density planting systems. Limited studies are available on the effect of planting density on romaine lettuce cultivars in controlled environment hydroponic system.

1.3 Objectives

1. Determine the yield plateau for Valley Heart lettuce in a controlled environment hydroponic system. By identifying the point at which increasing planting density no longer significantly increases crop yield, growers can optimize resource allocation and improve profitability.
2. Study the relationship between internodal elongation and the head formation in romaine lettuce to understand the effect of planting density on bolting.
3. Investigate the cultivars Breen and Valley Heart lettuce for yield and morphological characteristics in various spacing conditions (6.3 cm, 7.6 cm, 8.8 cm, 10.1 cm) and different planting patterns (staggered and non-staggered).

Connecting text

This following chapter provides a summary of hydroponic crop production in a controlled environment condition, highlighting various types of soilless farming techniques. It discusses factors which are crucial for indoor lettuce production, with particular focus on planting density effects on plant yield, morphology, and hormones.

Chapter 2 Literature review

2.1 Controlled Environment Agriculture (CEA)

The world population is growing and is estimated to reach 9.73 billion by 2050 and 11.2 billion by 2100 (Food and Agriculture Organization (FAO), 2017). In contrast, the world's population in rural areas has declined from 66.4 % of the world's population in 1960 to 46.1 % in 2015, with urban dwellers outnumbering rural residents in 2007. The projected world population in urban spaces is expected to increase to 68 % by 2050. The trend towards urbanization necessitates an increase in the rate of food production using available farmland (Food and Agriculture Organization (FAO), 2017; Ragaveena et al., 2021; United Nations 2014; United Nations, Department of Economic and Social Affairs, Population Division, 2019). The rapid growth and urbanization of the global population have resulted in an elevated consumption rate, while the world's resources, such as land, water, and minerals, are not matching this increase. Approximately 38.6 % of the glacier-free water and 70 % of global freshwater sources have already been used for agricultural production (Sacks, 2020; World Water Assessment Programme, 2009). Concurrently, the decline in agricultural land per capita is a rising threat to world food production, and the United Nations (FAO) has reported a reduction in arable land per person due to various phenomena such as climate change, lack of freshwater availability and population explosion (Fedoroff, 2015; Food and Agriculture Organization (FAO), 2016). Available arable land is additionally restricted by soil degradation and erosion, which results in the abandonment of cultivable land due to soil deterioration (Fischer et al., 2012). It is estimated that global food production may be reduced by 12 % by 2040 due to soil degradation (Kopittke et al., 2019; Noel, 2015).

To feed the rising population, we need to develop a more sustainable approach to food production that reduces greenhouse gas emissions, which are key critical contributors to climate change (Barbosa et al., 2015; Change, 2014). According to Food and Agriculture Organization (FAO) (2017), "the key to sustainable agricultural growth is a more efficient use of labor, and other inputs through technological progress, social innovation, and new business models".

CEA offers more sustainable production under distinct environmental conditions for high-density crop production (Bayley, 2020), and it is advantageous over conventional crop production, which is continually challenged with fluctuating weather, field pests, and pathogens, as well as varying soil and water conditions that act as a barrier for continuous production. CEA facilitates increased food production in small areas, thereby increasing land use efficiency. With conventional farming, crop production is limited to a horizontal area, which is a substantial limitation for the farming community. CEA can take advantage of the available vertical space, and ‘vertical farming’ offers considerably more production volume. In addition, environmental impact is lessened in CEA compared to field cultivation (Stanghellini, 2013). CEA facilitates the monitoring and control of environmental factors such as air temperature, water temperature, relative humidity (RH), and light intensity. Advanced technology and sensor systems may be used to monitor hydroponic systems in real time, producing a large amount of data that can be analyzed to optimize nutrient solutions and fertilizer inputs, in addition to increasing yield efficiency (Ragaveena et al., 2021). Soilless crop cultivation in CEA offers added benefits as a protected cultivation technique with fewer pesticides (Benke and Tomkins, 2017; Tajudeen and Taiwo, 2018).

2.2 Classifications of growing techniques in controlled environment agriculture

In CEA soilless farming has emerged as a popular method of growing crops. Soilless farming involves growing plants without soil, in a nutrient-rich water solution or other growing medium (Sharma et al., 2018). There are three main forms of soilless farming: hydroponics, aeroponics, and aquaponics (Alshrouf, 2017; Figure 1).

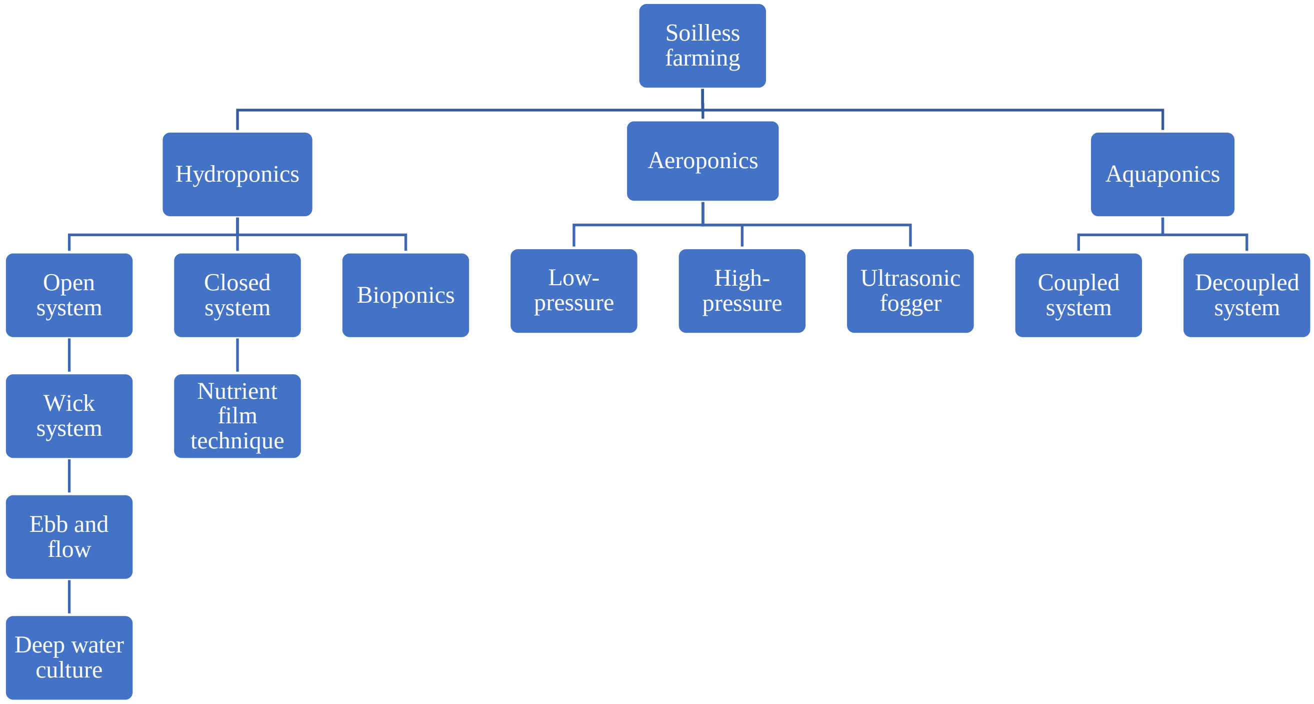

Figure 2.1 Classification of soilless farming techniques.

2.2.1 Aquaponics

The practice of raising fish and cultivating aquatic plants can be traced back to early Chinese societies that thrived before 1000 BC (Nash, 2010). In this system, plants absorb nutrients that come from fish waste or the microbial decomposition of organic matter. As a result, the organic matter is effectively broken down, facilitating the availability of essential nutrients for plant growth (Goddek et al., 2019). Microbes plays a crucial role in breaking down dissolved nutrients from fish excrement and unconsumed feed. Aquaponics is a sustainable approach that ensures net zero waste discharge from the system, thereby ensuring an eco-friendly approach that solves the issue of nutrient-rich runoff from traditional soil-based agriculture (Goddek et al., 2019).

2.2.2 Aeroponics

The term aeroponics is derived from two words: "aero," which is derived from a Latin word for "air," and "ponic," which is derived from the Greek word "ponos," meaning labor (Stoner and Clawson, 1998). Aeroponics is a type of soilless cultivation technique in which the plant roots are suspended in the air, exposing them to a nutrient mist in order to encourage optimal plant growth rather than relying on nutrient solutions or solid media, which are mostly used in traditional cultivational methods (Peterson and Krueger, 1988). In aeroponics, plants are suspended in an enclosed chamber and supported by plastic panels or polystyrene. The system includes pipes, spray nozzles, a pump, and a timer to distribute the nutrient solution from the storage tank to the plant root system (Goddek et al., 2019; Ragaveena et al., 2021). In aeroponic systems, a gas-dispersal mechanism accurately delivers nutrients to individual plant roots through a mist created by a sprayer. Nutrient cycles are maintained by a timer to prevent root desiccation. Aeroponics is further classified into different types based on method used to deliver nutrients and water to the plant roots (Figure 1). In low pressure aeroponics, the nutrient delivery is carried out by low pressure pump which sprays the nutrient solution at a droplet size around 100 µm (Wainwright et al., 2004), while the high-pressure systems produces a pressure around 80-100 psi (Lakhiar et al., 2019) which can deliver the mist at a droplet size of 45-55 µm (Gonyer and Jones, 2016). Meanwhile, the ultrasonic foggers create high-frequency waves to produce a mist layer which floats in the air to form a thick fog cloud (Lakhiar et al., 2018). Aeroponics has the potential to decrease water usage by 98 % and fertilizer usage by 60 %, thereby resulting in yield maximization of up to 45 to 75 % when compared to conventional soil-based farming (NASA, 2006).

2.3 Hydroponic crop cultivation

Hydroponic cultivation in CEA assures soilless food production irrespective of outdoor weather conditions (Barbosa et al., 2015). Hydroponics is derived from the English suffix hydro- "of water," and Greek word ponos – "labor," (Beibel, 1960; Khan, 2018). Through hydroponics, plants are grown using nutrient-filled irrigation water instead of soil to fulfill plants nutritional requirements (Bridgewood, 2002; Hochmuth and Hochmuth, 2001; Khan, 2018; Tyson et al., 2001). Plants are grown directly in the nutrient solution or may be supported by an inert medium (aggregate culture) (Kaiser and Ernst, 2012). The plants nutritional requirements in both systems are fulfilled by irrigation water supplemented with nutrients. Plants grown directly in solution have an advantage over those raised in a medium because their roots are constantly exposed to the water and nutrient solution (Butler and Oebker, 1962). A comparative study conducted by Barbosa et al. (2015) resulted that hydroponically grown lettuce demonstrated higher yield potential (41 kg m^{-2} $year^{-1}$) than the lettuce grown in soil (3.9 kg m^{-2} $year^{-1}$). Hydroponics has the potential to provide food for millions in regions where water and crop production is limited (Nabi et al., 2022). Hydroponic cultivation produces fewer soil particles, eliminating most of the soil-borne pest and disease incidence, the need for washing, and reducing water and energy usage (Serio et al., 2001; Verdoliva et al., 2021). Shifting the growth medium is viable for long-term crop production and the conservation of rapidly dwindling land and water resources (Sharma et al., 2018). While some forms of traditional soil cultivation leads to pollution of land and water resources, both can be eliminated in a closed hydroponic system (Bar-Yosef, 2008; Carmassi et al., 2005; Verdoliva et al., 2021)

Both the qualitative and quantitative performance of crops is related to the irrigation system (Dukes et al., 2010). Poor crop yield and quality are the results of insufficient irrigation, while over-irrigating the land leads to a waste of irrigation water while making the crop vulnerable to disease (Pardossi et al., 2009). To ensure resource sustainability and to decrease the environmental impact of irrigation, we need an effective watering system (Kadirbeyoglu and Özertan, 2015).

Of all the soilless agriculture techniques (Figure 2.1) hydroponics is becoming more popular in CEA due to effective resource management and food production, further ensuring uninterrupted crop production throughout the year, higher crop yield and water efficiency (Brechner, 2014). Commercial producers use hydroponic cultivation to grow tomatoes and leafy greens such as lettuce, cilantro, and spinach. Lettuce is well known for its excellent performance in hydroponic systems (Tognoni and Pardossi, 2020), accounting for up to 23.5 t ha^{-1}, compared to an open agricultural yield of 10 t ha^{-1} which is a 233 % rise (Singh, 2012). A study conducted by Barbosa et al. (2015) concluded that hydroponic lettuce cultivation resulted in 11 times increased yield and uses 13 times less water than conventional farming methods. There are several types of hydroponic systems, such as the nutrient film technique (NFT), the ebb and flow technique, the wick system, and the deep water culture (DWC) (Stone, 2014) (Figure 2.1). In this thesis, ebb and flow has been chosen for its ease of use and efficient nutrient & water management.

2.3.1 Ebb and flow systems

Ebb and flow hydroponic system, also known as the flood and drain system, comprises of a setup with growth media on the plant tray above the nutrient reservoir from which the solution is pumped with the aid of submersible pump controlled by a timer (Ernst, 2009; Van Patten, 2004). Capillary action is employed in ebb-and-flow irrigation, sub-irrigating potted plants to limit nutrient leaching from the growing medium, reducing water usage and increasing yield for crop production (Dole et al., 1994; Neal and Henley, 1992; Poole and Conover, 1992; Thomas, 1993). Sub-irrigation using ebb and flow reportedly saves 86 % of water compared to overhead irrigation (Ahmed et al., 2000). Irrigation and nutrient supply can be easily modified in ebb and flow systems to best respond to the plants specific needs, preventing excess watering and nutrient(Ferrarezi et al., 2015). For lettuce production, ebb and flow resulted in a 33 % reduction in water consumption and improved water use efficiency by 20 % compared to traditional overhead watering (Yang et al., 2018). Furthermore, automation in fertigation aids in saving time and labor costs (Ferrarezi et al., 2015). Automated fertigation units ensure optimum delivery of water and nutrients at required frequency which are critical to optimal crop growth (Magán et al., 2001). Smart fertigation system ensures timely irrigation and fertigation which improves plant yield and development by reducing occurrence of plant stress due to irregular irrigation and excessive fertigation (Karaşahin et al., 2018).

2.3.2 Bioponics

The National Organic Standards Board (NOSB) introduced the term ‘Bioponics’ in 2016. Bioponics is a soilless agriculture technique that obtains nutrients from natural substances like plant-based, animal-based, and mineral sources (Fang and Chung, 2017). These substances are completely degraded and broken down by microorganisms, which releases nutrients to the plants in this system (Wongkiew et al., 2021). Bioponics offers the advantages of both organic soil-based agriculture (utilization of reclaimed resources) and hydroponics (autonomous growth system with precise control over plants) without the associated disadvantages (high energy and maintenance cost). Using organic compost technology to extract nitrogen from different organic waste streams enables sustainable production of high-quality crops throughout the year (Beacham et al., 2019; Lee and Lee, 2015; Prabhas et al., 2018; Singhania and Singhania, 2014).

2.4 Hydroponic nutrient solution

Essential minerals for greenhouse crops grown in hydroponic systems are provided by a nutrient solution enriched with set concentrations and ratios of water and minerals suitable for plant growth (Karimaei et al., 2001). The concentration of mineral salts is higher in hydroponic systems when compared to soil-grown crops, while heavy metal concentrations are lower (Massantini et al., 1988). Tailoring nutrient solutions for specific crops is commonly practiced. Crops like spinach and lettuce tend to accumulate nitrate in their leaves by omitting these ions in their final growth phase; this can be effectively eliminated by the proper operation of a hydroponic system (Karimaei et al., 2001).

Woodward (1699) was the first known to employ solution culture techniques for examining the growth of terrestrial plants (Addoms, 1937), while (De Saussure, 1804) was the first to develop nutrient solutions by dissolving various salts in distilled water (Asher and Edwards, 1983). In 1842, German botanists Julius von Sachs and Wilhelm Knop shortlisted nine essential nutrients for plant growth which later led to the development of soilless cultivation from 1859 to 1875 (Douglas, 1975). Sach (1860) later proposed a standard formula for nutrient solution that is widely used to grow plants successfully without an inert media (Table 2.1). Nutrient solutions with different chemical constituents have since been suggested by Tollens (1882), Schimper (1890), Pfeffer (1900) , Crone (1902), Tottingham (1914), Shive (1915), Hoagland (1920), and many others Hoagland and Arnon (1950) (Table 2.1). These solution concentrations are still employed in laboratory research and hydroponic studies.

Table 2.1 Composition of nutrient solution used by other researchers. (Hoagland and Arnon, 1950; Miller, 1938)

Sach's solution (Sachs, 1887)		Knop's solution (Knop, 1865)		Pfeffer's solution (Pfeffer, 1900)		Crone's solution (Crone, 1902)		Hoagland's solution (Hoagland and Arnon, 1950)	
Chemical constituents	g l^{-1} in H_2O	Chemical constituents	g l^{-1} in H_2O	Chemical constituents	g l^{-1} in H_2O	Chemical constituents	g l^{-1} in H_2O	Chemical constituents	g l^{-1} in H_2O
$Ca_3(PO_4)$	0.50	$Ca (NO_3)_2$	0.8	$Ca (NO_3)_2$	0.8	KNO_3	1.00	KNO_3	101.10
KNO_3	1.00	KNO_3	0.2	KNO_3	0.2	$Ca_3(PO_4)_2$	0.25	$CaNO_3$	236.15
$MgSO_4$	0.50	KH_2PO_4	0.2	$MgSO_4$	0.2	$MgSO_4$	0.25	$MgSO_4$	246.50
$CaSO_4$	0.50	$MgSO_4$	0.2	KH_2PO_4	0.2	$CaSO_4$	0.25	$KHPO_4$	136.09
NaCl	0.25	$FePO_4$	Trace	KCl	0.2	$FePO_4$	0.25	H_3BO_3	2.860
					Small			$Mncl_2.4H_2O$	1.810
$FeSO_4$	Trace			$FeCl_3$	amount				
								$ZnSO_4.7H_2O$	0.220
								$CuSO_4.5H_2O$	0.80
								$Na_2Mo_4.2H_2O$	0.120
								Na EDTA	7.450
								$FeSO_4.7H_2O$	5.570

The most used growth solution for plants is Hoagland's nutrient solution (Cramer and Spurr, 1986; Hoagland and Arnon, 1950; Lee et al., 1981; Paiva et al., 1998). Hoagland developed two types of hydroponic solutions (Hothem et al., 2003; Hoagland and Arnon, 1950;). For the first solution, the entire nitrogen supply for the plants was provided in the form of nitrate. For the second solution, ammonium was used instead of nitrate as a nitrogen stock to maintain pH at lower levels. Iron tartrate was added to both solutions as an iron source. In the present-day scenario, this has been modified by adding iron chelates such as Fe-EDTA, Fe-DTPA, or Fe-EDDHA (Hothem et al., 2003). Hoagland's solution is the most suitable nutrient solution for soilless lettuce production (Massantini et al., 1988).

2.5 Lettuce origin and distribution

Lettuce is a leafy green that can be cultivated successfully in a controlled environment hydroponic system (Kang et al., 2013).The term lettuce is used to refer to the edible, succulent leaves of *Lactuca sativa* (Katz & Weaver, 2003). Lettuce belongs to the class Magnoliopsida, order Asterales, and the flowering family Asteraceae. Today, lettuce is one of the most commonly used leafy greens in salad mixtures and sandwiches (Mou, 2008) and is the most widely consumed leafy green in the world (Kim et al., 2016). Evidence portrayed on Egyptian murals of long-leafed lettuce dating back to 4500 B.C. suggests that lettuce originated from the Mediterranean region., slowly spreading to the nearby areas of Greece and Rome (Lindqvist, 1960). The Greek historian Herodotus mentioned that they cultivated cos-like lettuce consumed at the Persian court (De Vries, 1997). Modern-day lettuce is a direct descendant of the common wild lettuce variety (*Lactuca serriola)* (De Vries, 1990, 1997; Kesseli et al., 1991; Lindqvist, 1960), which is distributed in regions where modern-day lettuce is cultivated. Mou (2008) suggests that genetic mutations in *Lactuca serriola* led to the development of favorable plant characteristics for human consumption. Eventual plant breeding programs and variety selection promoted desired physical characteristics such as size, shape, color, texture, head formation, and taste while eliminating undesired characteristics (i.e. thorns in stem and leaf rinds, bitterness present in leaf tissues and reduced leaf latex content) present in wild lettuce (Mou, 2008). Recently, lettuce was classified into six major types based on leaf shape, size, texture, head formation, and stem type. They are (1) crisp head lettuce (cv. capitata), (2) butterhead lettuce (cv. capitata), (3) romaine or cos lettuce (cv.

longifolia), (4) leaf or cutting lettuce (cv. acephala), (5) stem or stalk (asparagus) lettuce (cv. angustana, cv. asparagine), and (6) Latin lettuce (cv. little gem) (Mou, 2008).

2.6 Greenhouse lettuce production

Greenhouse crop production allows for food production during adverse external environmental climates. Hickman (2016) defines a greenhouse as “a structure designed for the cultivation of plants to protect against extreme environmental conditions and pests”. Production of leafy greens in a controlled environment is assumed to be more protected from microbial contamination when compared to the field conditions where pre-harvest contamination often occurs because of wild animals, pests, human interference, and soil contamination (Holvoet et al., 2015). Application of fertilizer and pesticides accounts for the contamination of leafy greens grown in the field (Food and Drug Administration, 2008; Franz and van Bruggen, 2008; Gu et al., 2013; Horby et al., 2003; Islam et al., 2005; Suslow et al., 2003). Present-day greenhouses primarily focus on increasing crop yield by enabling 365-day crop production within an optimized environment for plant growth (Xia, 2019). When grown in a controlled environment, growth, development, and plant yield are ultimately determined by environmental parameters (Hiroki et al., 2013), and lettuce is one of the most commonly produced crops under controlled environment conditions (Bian et al., 2018).

2.7 Controlled environment conditions for lettuce growth

Essential considerations for hydroponic lettuce growth and development in CEA are light, temperature, humidity, nutrient solution, pH, electrical conductivity (EC) of the nutrient solution, and dissolved oxygen (DO) concentration in the nutrient solution (Gent, 2017). Lettuce is a cool season crop well known for germination at lower temperatures, optimally at an average temperature of 18 °C (Lafta and Mou, 2013). This crop is well known for its rapid growth rate, short maturation period, and suitability for high-density planting conditions, demanding less energy (Bantis et al., 2018). Climatic regions with prolonged winters and low photoperiods prominently affect lettuce production, but this can be overcome with supplemental light and a temperature control system in the greenhouse. Lettuce heads grown in soil are harvested by cutting them at the base and leaving the root residues in the soil, while lettuce grown in controlled environment hydroponic systems can be harvested with intact roots, ensuring fresh produce for more than two weeks when refrigerated (Chidiac, 2017; Couture et al., 1993). To produce higher lettuce yield, it is essential to maintain the optimal environmental conditions that allow efficient use of available resources in a controlled environment (Ahmed et al., 2020b).

2.7.1 Air and leaf temperature

Air temperature impacts plant growth and development directly and indirectly. Plant metabolic processes such as photosynthesis, transpiration, and respiration are directly influenced by temperature. The absorption of water and fertilizer in plants is indirectly influenced by the air temperature (Ahmed et al., 2020b; Tian et al., 2014). Higher air temperature causes an increase in the plant's evapotranspiration, leading to increased water absorption, while lower air temperature can reduce water movement and absorption in plants (Arndt, 1937; Clements and Martin, 1934; Delf, 1916; Jensen and Taylor, 1961; Kramer, 1940, 1942). Under controlled environmental conditions, one of the ways to produce an ideal lettuce head is by providing and maintaining an ambient temperature (Choi et al., 2011).

According to Decoteau (2000), ideal crisp head lettuce heads are obtained with a maximum mean day temperature of 23 °C and a minimum mean night temperature of 7.2 °C. This lettuce tends to have a reduced vegetative phase and rapid bolting conditions when exposed to higher temperatures that eventually lead to improper head formation, which is not economically viable for growers (Nagai and Lisbão, 1980; Whitaker et al., 1986). Decoteau (2000) further reported loose head formation with a bitter taste due to higher temperatures. However, lettuce head growth is inhibited with a prolonged lower night temperature (Dufault et al., 2009). A study by Choi et al. (2000) suggests that maintaining the temperature at 30/25 °C positively affects crisp head lettuce by increasing the photosynthetic activity of the crop. However, a reduced temperature of 20/15°C is recommended for improving the lettuce's photosynthetic rate at later stages. Knight and Mitchell (1983) conducted a study on the stimulation of lettuce productivity by manipulating diurnal temperature and light. The study revealed that higher growth rates for lettuce were observed at 25/25 °C day/night temperatures while higher lettuce dry mass was obtained at 24/24°C in the greenhouse. Ahmed et al. (2020b) conclusively suggests an optimum air temperature of 22–25 °C and 18–20 °C during the day and night, respectively, for lettuce production in a controlled environment.

2.7.2 Leaf temperature

The transpiration rate for a plant grown in a controlled environment is primarily determined by leaf and air temperature (Ahmed et al., 2020a; Jones and Rotenberg, 2001). As water evaporates from the stomata, a negative suction pressure is created in the plant's xylem tissues, enabling water and nutrient uptake through plant roots (Ahmed et al., 2020b). In a controlled environment for lettuce production, a reduced plant transpiration rate may lead to tip burn in inner heart lettuce leaves (Goto and Takakura, 1992a, b; Sago, 2016; Saure, 1998; Zhang et al., 2016). Water and nutrient uptake rate in romaine lettuce can be enhanced by increasing the heat and mass transfer rate, which aids in eliminating tip burn (Ahmed et al., 2020a).

Lettuce leaf and air temperatures are generally intertwined in determining lettuce yield in a controlled environment. Leaf development rate is determined by ambient air temperature (Karlsson and Werner, 2001), whereas plant growth and development are determined by leaf temperature more so than air temperature (Fujiwara et al., 2004; Hatfield and Prueger, 2015; Kaiser et al., 2015). Leaf enzyme activity is impacted by air temperature (Bernacchi et al., 2001; Florian et al., 2014; Kumarathunge et al., 2019; Timm et al., 2019; Walker et al., 2013), affecting plants during growth and development stages, ultimately determining the crop yield (Ruiz-Vera et al., 2018; Zhu et al., 2018). For rapid plant growth and higher accumulation of biomass, there is a need to maintain an optimal air temperature (Kaiser et al., 2015; Tian et al., 2014), while the light source and rate of airflow are environmental phenomena that can alter both air and leaf temperature in a controlled environment. Air velocity attributes to the temperature difference between the leaf and air temperature, and the continuous emission of thermal radiation from the light source contributes to the temperature difference between air and the leaf (Ahmed et al., 2020b). A study conducted by Kitaya et al. (1998) reported that eggplant (*Solanum melongena*) grown under high-pressure sodium (HPS) light exhibited a higher leaf temperature of 1 °C than the air temperature, as HPS has a higher thermal emission of radiation than fluorescent lamps. Therefore, light source and intensity should be carefully selected to maintain an optimum air temperature.

2.7.3 Air velocity

Air velocity determines latent heat exchange by regulating plant heat exchange and transpiration rate (Bonan, 2015). Air temperature, RH, and CO_2 concentrations at the plant canopy level are influenced by air velocity. The exchange of CO_2 and conductance of water vapor in the plant systems is regulated by the stomatal conductance mechanism, which controls both transpiration and photosynthesis activities carried out by the plants (Tuzet, 2011). A study by Korthals et al. (1994) concluded that higher air velocity could cause stomatal closure, reducing photosynthetic rates and plant growth. Similarly, lower air velocity causes a reduction in plant photosynthetic rates due to lower CO_2 exchange rates, ultimately leading to an increased thickness of the leaf layer boundary (Nobel, 1981). Therefore, there is a need to maintain an optimum air velocity that promotes the growth of plant canopy by stimulating gas exchange (Ahmed et al., 2020b; Kitaya et al., 2003; Korthals et al., 1994) and this can be based on the photosynthetic and transpiration rates of a given plant species (Chintakovid et al., 2002). Plant structure, plant canopy depth, and airflow direction should further be considered (Kitaya et al., 2003; Sase, 2006; Shibuya and Kozai, 1998). According to Ahmed et al. (2022), fresh mass is impacted by air velocity and airflow direction in CEA. (Ahmed et al., 2020b; Kitaya et al., 2004) suggests 0.3-0.7 m s^{-1} as an optimum velocity for tomato (*Lycopersicon esculentum* cv. Momotaro) cultivation in controlled environment condition. Nishikawa et al. (2013) studied the effect of air velocity on the growth response of lettuce (cv. Greenwave) seedlings. With rotation, the experiment was performed with three different air velocities, 1.8, 0.9, and 0.1 m s^{-1}. Lettuce growth was enhanced by 20 % in lettuce when grown under an air velocity of 0.9 m s^{-1} with rotation. At an air velocity of 0.9 m s^{-1}, lettuce fresh and dry mass increased by 17.3 % and 8.7 %, respectively, compared to lettuce grown at an air velocity of 0.1 m s^{-1}. A study by Lee et al. (2013) with 28 different lettuce cultivars concluded that increasing the horizontal air velocity in a closed plant factory to 0.28 m s^{-1} or higher decreased tip burn symptoms. The same study suggests that a higher air velocity of 1.04 m s^{-1} resulted in reduced plant growth. Ahmed et al. (2022) investigated the effect of different air velocities (0.25, 0.50, and 0.75 m s^{-1}) on the growth of lettuce. The study concluded that the incidence of tip burn in lettuce was reduced by 87.3 % when air velocity was increased from 0.25 to 0.75 m s^{-1}. While a study conducted by Shibata et al. (1994) on lettuce (cv. Red-fire) found that using a vertical air flowing system with an air velocity of 0.7 m s^{-1} resulted in reduced resistance of CO_2 and H_2O diffusion without closing the stomata thereby increasing the lettuce yield up to 130 % when compared to the horizontal air flowing system.

2.7.4 Relative humidity (RH)

Relative humidity is a crucial environmental factor that can impact the growth of plants in a closed chamber. It represents the amount of water vapor present in the air as a percentage of the maximum amount the air could hold at a given temperature. The percentage of relative humidity can vary depending on the air temperature and the level of evaporation occurring in the chamber (Bramer et al., 2018; Chia and Lim, 2022). It directly regulates water loss by transpiration and stomatal opening. RH indirectly influences water potential, photosynthesis, nutritional translocation, and plant temperature (Tibbitts, 1979). The most precise way of measuring RH in a controlled environment is by vapor pressure deficit (VPD), which involves the loss of water from a leaf due to the pressure difference between the plant and the surrounding environment (Gómez et al., 2019). Relative air humidity is essential in adapting the stomata's response to CO_2 (Talbott et al., 2003), and VPD directly influences stomatal conductance.

Stomatal conductance decreases at a high VPD, thereby increasing the evapotranspiration rate, eventually leading to excessive moisture loss (Ahmed et al., 2020b; Kaiser et al., 2015). Low vapor pressure deficits reduce plant transpiration, decreasing plant metabolism rate and development (Ngo et al., 2013). Maintaining an optimal RH that facilitates an optimum vapor pressure deficit within the plants is important. The ideal level of humidity changes according to plant species and environmental conditions (Ahmed et al., 2020b; Mortensen and Gislerød, 1990). A study by Shibata et al. (1994) reported that an RH of 60 % induced water stress in CEA-grown lettuce. When grown at 80 % RH, lettuce showed higher gas exchange rates that promoted plant growth and yield. Tibbitts (1976) reported that lettuce grown at 85 % RH exhibited less stomatal resistance, promoting the photosynthetic rates per unit area of lettuce. The same study showed that lettuce grown in higher humidity (85 %) resulted in larger marketable heads with higher moisture content, increased leaf number, leaf size, and plant yield compared to lettuce grown at lower humidity levels (50 %). Four weeks after transplant, lettuce at higher humidity exhibited an increased growth rate, resulting in a greater fresh and dry mass of 60–100 % and 45–70 %, respectively (Tibbitts, 1976).

2.7.5 pH

Water quality is critical for vegetable production in CEA, and water alkalinity affects nutrient solution pH and directly influences plant growth and quality (Roosta, 2011). The availability and solubility of several nutrients, especially iron and phosphorus are determined by pH of the nutrient solution (Bugbee, 2003; Jones Jr, 1982). In a hydroponic system, salts like iron (Fe), manganese (Mn), calcium (Ca), and magnesium (Mg) may start precipitating if pH exceeds 7, making them unavailable to plants. A slightly acidic pH prevents nutrient unavailability, ensuring optimum hydroponic growth (Resh, 2004). Both pH and EC play an essential role in solubility, efficient absorption of minerals, and plant growth. Bres and Weston (1992) reported that a pH range of 5.0 to 6.5 was optimum for lettuce cultivars (‘Summer Bibb’ and ‘Buttercrunch’). According to the study conducted by Roosta (2011) nutrient solution with an acidic pH improved the overall lettuce growth for (cv. Parris Island). At this pH, maximum fresh and dry mass of roots and shoots were reported. This study revealed that higher pH levels of the nutrient solution caused a reduction in both iron concentration and lettuce leaf color. Doğan and Salman (2007) reported that the lettuce exhibited peak poly phenol oxidase (PPO) activity at a neutral pH level. Davies and Asker (1983) studied the effect of pH on the synthesis of oxalic acid from lettuce leaves and reported that alkaline pH favored oxalate enzyme synthesis.

2.7.6 Light

Light is paramount to lettuce grown in controlled environments (Singh, 2012). Light is the principal energy required for plant photosynthesis, and it promotes plant growth and development by influencing physiological processes (Bayat et al., 2018; Bian et al., 2015). Light stimulates various plant signals related to morphogenesis and plant physiological processes responsible for plant growth and development (Chen et al., 2004). Such processes are seed germination (Bentsink and Koornneef, 2008), the direction of plant growth (Pedmale et al., 2010), shoot elongation (Casal, 2013), leaf growth (Cookson and Granier, 2006; De Carbonnel et al., 2010), root growth (Sakamoto and Briggs, 2002), flowering (Alvarez-Buylla et al., 2010; Park and Runkle, 2017) and synthesis of plant pigments such as chlorophylls, carotenoids, and anthocyanins (Duchovskis et al., 2005; Dutta Gupta and Agarwal, 2017; Li and Kubota, 2009; Merzlyak et al., 2008; Ouzounis et al., 2014; Pizarro and Stange, 2009; Ruberti et al., 2012; Samuolienė et al., 2013). According to Pardo et al. (2013) high intensity light emitting diode (LED) (red, blue, and green) wavelengths had an effect on overall germination and average hypocotyl length of different lettuce varieties (Boston, Roman, Black Simpson). Wavelengths ranging from 400–700 nm, known as photosynthetic active radiation (PAR) and measured as photosynthetic photon flux density (PPFD) is crucial for plant photosynthesis (Gallo and Daughtry, 1986). To supplement natural light in a controlled environment (greenhouse, plant factory, tissue culture rooms), electrical light sources that emit photons with a spectrum ranging between 350–700 nm, such as fluorescent lights (FL), HPS, metal halide and incandescent (INC) lamps, which have been conventionally utilized over a long period of time. The most widely used lighting sources for lettuce production in plant factories are FL, HPS, and LEDs. (Bian et al., 2015; Shimizu et al., 2011; Zhang et al., 2018). According to Rácz (2012) potential cost reduction with compact fluorescent tubes and LED lamps can be attained, which varies based on the number of hours they are used each day, and can range from 26 % to 79 % when compared to traditional incandescent light bulbs. Meanwhile, FL have a wide range of wavelength between 350-750 nm such wide spectrum is not essential for production of certain plant species (Dutta Gupta and Agarwal, 2017). HPS lamps produce excessive heat that can change the air temperature, reducing energy efficiency. LEDs are growing in popularity, acting as an energy-efficient lighting system for plant production in plant factories (Singh et al., 2015). With LEDs, irradiance and the spectrum can be easily controlled (Lin et al., 2013).

2.7.7 Photoperiod

Photoperiod influences plant growth and development from germination to flowering (Singh et al., 2015). By adjusting the photoperiod, plants morphological and physiological features can be altered (Jackson, 2009; King, 2009; Valverde et al., 2004). Zhang et al. (2018) reported that in addition to light intensity and quality, plant nitrate content, vitamin C, soluble sugar, soluble protein, and anthocyanin concentration were significantly influenced by photoperiod. Soffe et al. (1977) reported that the plant dry mass and the head size of the lettuce (cv. Kolck) were doubled compared to the average yield when the photoperiod was increased from 12 h to 16 h. Bian et al. (2015) suggests that continuous illumination with red and blue LED light (R:B = 4:1) for 48 h before harvest would be the best way to maximize the hydroponic lettuce's nutritional content. Dorais (2003) reported that increasing the photoperiod for lettuce production from 16 h to 24 h resulted in increased biomass accumulation but adversely led to increased nitrate content in the leaves. The study conducted by Zhang et al. (2018) using red-blue LEDs at 250 µmol m^{-2} s^{-1} and a 16 h photoperiod promoted optimal lettuce yield, while Loconsole et al. (2019) reported that an average photoperiod of 16 h day^{-1} and light intensity of 165 µmol m^{-2} s^{-1} comprised of red-blue LEDs resulted in optimal romaine lettuce plants in terms of yield and quality. Another study suggests 16 to 18 h as an optimum photoperiod for increasing lettuce growth rate, reducing nitrate accumulation, and improving light use efficiency (Ahmed et al., 2020b). Shao et al. (2020) conducted a study to investigate the effect of high light radiation (500 µmol m^{-2} s^{-1}) in the middle of the light period (150 µmol m^{-2} s^{-1}, 16 h photoperiod) to determine its effect on biomass accumulation of lettuce (cv. Zishan); illuminating lettuce with high light intensity for 1 to 2 h in between the light period promoted fresh mass, dry mass and nutritional quality of the lettuce (Kuno et al., 2017) A study by Kuno et al. (2017) investigated the impact of red-blue LED irradiation with a light intensity of 120 µmol m^{-2} s^{-1} and varying photoperiods on the growth of leafy lettuce (cv. Green wave); illuminating lettuce for 12 h day^{-1} resulted in a significant increase in both fresh and dry mass of the lettuce plants. Lee and Kim (2013) reported that maintaining a light intensity at 230 µmol m^{-2} s^{-1} increasing the photoperiod from 16 h to 20 h day^{-1} promoted growth rate, efficient usage of energy, and reduced leaf nitrate content.

2.8 Tip burn

In controlled environment lettuce production, tip burn is the most significant physiological disorder responsible for reduced quality and economic yield (Ahmed et al., 2020b). Tip burn is associated with the development of necrotic lesions on the margin of leaves, including the inner leaves found inside the lettuce head (Macias-González et al., 2019). This frequently occurs before harvesting due to stagnant stomatal boundary leading to a reduced transpiration rate (Lee and Kim, 2013; Zhang et al., 2016). Localized calcium deficiency during leaf growth is linked to tip burn in lettuce (Barta and Tibbitts, 1991), and the movement of calcium from the plant roots to the developing lettuce leaves is accelerated by increased plant transpiration (Both, 2002). Higher RH (Barta and Tibbitts, 1991; Choi and Lee, 2008; Frantz et al., 2004) and other rapid lettuce growth-promoting conditions such as temperature and high nitrogen supply (Brumm and Schenk, 1992; Misaghi and Grogan, 1978) are vital contributors to tip burn occurrence (Macias-González et al., 2019). Tip burn incidence is additionally influenced by the lettuce cultivar (Choi et al., 2000; Sago, 2016) and incidence in lettuce can be reduced by deploying tip burn-resistant cultivars (Olle and Bender, 2009; Ryder and Waycott, 1998). According to Read and Tibbits (1970), an increased growth rate resulted in accelerated tip burn development in lettuce (cv. May Queen), primarily caused by increased light intensity and CO_2 concentration. Enhanced airflow in CEA helps prevent the occurrence of tip burn in lettuce plants (Goto and Takakura, 1992a, b; Saure, 1998; Zhang et al., 2016). Both (1995) reported that a plant transpiration rate of 400 mL g^{-1} dry mass lettuce (cv. Ostinata) prevented the incidence of tip burn. Increased airflow improves lettuce's transpiration rate, thereby enhancing calcium's efficient transport to newly developed inner leaves and reducing tip burn occurrence (Choi et al., 2000; Lee and Kim, 2013; Shibata et al., 1994). Both (2002) investigated the effect of day light intensity (DLI) on tip burn occurrence and reported that lettuce (cv. Butterhead) grown under daily integrated light levels of 17 µmol m^{-2} s^{-1} did not have any tip burn. Likewise, vertical airflow towards the lettuce reportedly increases yield by up to 30 % and reduces the incidence of tip burn (Shibata et al., 1994). Increasing the amount of blue light in the spectrum improves calcium levels in lettuce leaves, offering a potential mitigation strategy for tip burn (Mickens et al., 2018; Pennisi et al., 2019). Saure (1998) demonstrated that tip burn was not reduced by soil or foliar calcium applications. Hartz et al. (2007) investigated the effect of calcium fertilizer application through a drip irrigation system for romaine lettuce (cv. longifolia) and reported that only the concentration of calcium in lettuce leaves was high and it had no effect on tip burn incidence. Bárcena et al. (2019) studied the impact of shade cloths and polyethylene covers on tip burn incidence in greenhouse-grown lettuce (cv. Longifolia Lam. Crimor-INTA). This study revealed that all the plants grown under polyethylene were affected by tip burn, while plants grown under shade cloths had none.

2.9 Correlation between biomass and planting density

The analysis of plant competition begins with assessing how plant growth and performance are influenced by the number of plants per unit area (Bleasdale, 1967; Shinozaki, 1956; Weiner and Freckleton, 2010; Yoda, 1963). When assessing a plant population, it is critical to evaluate not only the number of plants per unit area but also the distribution and arrangement of these plants within that area (Willey and Heath, 1969). According to Holliday (1960), there are two main types of relationships between biomass and planting density, asymptomatic and parabolic relationships. The former is a relationship where yield increases as density increases and reaches a maximum, but then it remains relatively constant at higher densities. For the latter, the yield increases as density increases and attains a maximum but decreases at higher densities.

According to (Harper, 1977; Shinozaki, 1956; Weiner and Freckleton, 2010), as the plant density increases, the amount of biomass per unit area initially increases proportionately but eventually, reaches a plateau where it no longer rises, thereby attaining constant final yield (Figure 2.1a). Planting density can be classified into three stages (Duncan, 1986). During the initial stage, there is no minimal competition between the developed plants, and the yield per unit area is primarily determined by the rate of light interception. This stage is classified as the stage 1 of the planting density (Duncan, 1986). As the number of plants per unit area increases between stages 1 and 2, mutual shading among the plants reduces the rate of light interception, resulting in increased competition for light. However, the total light intercepted per unit area increases in this phase, leading to an increase in yield per unit area but reduction in individual plant yield. This yield increase per unit area is attributed to both an increase in light interception and an increase in light utilization efficiency. In stage 3, the yield per unit area reaches a peak for the specific cultivar and the environment provided, meanwhile lodging and mortality increases between the individual plants (Figure 2.1a, 2.1b & 2.1c). The effect of plant density can vary according to the plant species. Different plant species have unique growth habits, root systems, and light requirements, which influence how they respond to changes in plant density (Allen, 1978).

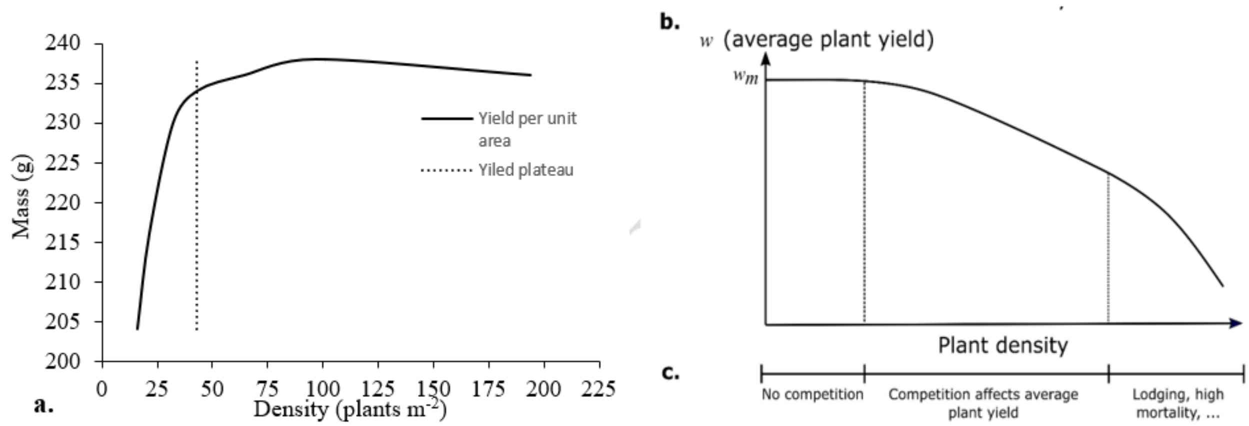

Figure 2.1 Relationship between plant yield and plant density in soybean (*Glycine max*) (Figure 2a) redrawn from (Wiggans, 1939). The maximum yield per plant (w) is affected by plant density, where W_m represents the yield of a single plant in isolation. The factors that impact total grain yield and average plant yield vary based on the density of plants (Beaugendre et al., 2022; Weiner and Freckleton, 2010).

2.9.1 Lettuce plant density

Very few studies exists on plant population density, which is correlated with plant intra-inter-row spacing, affect crop output, duration, and quality (Nawaz et al., 2021). To cultivate a viable plant population and increase yield output, plant row spacing plays a critical role (Bednarz et al., 2005). As planting density can significantly impact the crop yield, it is crucial in deciding production volume (Bayley, 2020). To maximize lettuce yield potential, it is critical to understand crop response to yield potential. The distance between the leafy lettuce heads in a hydroponics system directly influences the growth and yield potential (Maboko and Du Plooy, 2009). Traditional crop spacing adapted for romaine lettuce is usually 0.25–0.36 m for intra-row spacing and 0.41–0.61 m for inter-row spacing, estimating up to 4.6 to 9.7 plants m^{-2} (Knott, 1957). According to Chu et al. (2016), optimum lettuce row spacing was 0.24 m–0.32 m (5.1 – 10.4 plants m^{-2}) for the CEA facility located in California.

Maboko and Du Plooy (2009) studied the effect of plant density on different varieties of leafy lettuce raised in a gravel-filmed hydroponics system, with the hypothesis that increasing spacing between romaine lettuce plants would increase yield per unit and reach a yield plateau while decreasing spacing would increase individual plant mass to attain a mass plateau. The study concluded that greater spacing between plants improved individual plant fresh mass, dry mass, number of leaves, leaf area, and average plant height. Increasing the plant spacing reduced the yield per unit area (Figure 2.2). Khazaei et al. (2013) investigated the effect of spacing on iceberg lettuce growth and development and reported a yield plateau for iceberg lettuce at a plant spacing of 40 × 25 cm.

Bayley (2020) conducted a study on the effect of different plant spacings on romaine lettuce in controlled environment hydroponic system. This study revealed that a planting density of 30 plants m^{-2} was recommended to attain the highest overall yield of lettuce grown under a controlled environment hydroponic system. In contrast, a planting density of 10 plants m^{-2} was recommended for achieving the highest individual lettuce biomass. The authors concluded that the plant yield and morphology of romaine lettuce were determined by planting density.

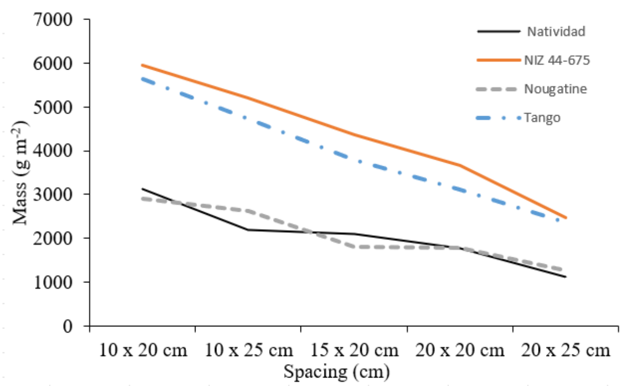

Figure 2.2 Yield response of different lettuce cultivars in various plant spacings adopted from Maboko and Du Plooy (2009).

2.10 Effect of planting density on lettuce morphology

Numerous studies have examined the impact of plant density on plant morphology and physiology of lettuce (Khazaei et al., 2013; Maboko and Du Plooy, 2009). Bayley (2020) studied the effect of plant density on the yield and quality of romaine lettuce in a controlled environment hydroponic system. Reduced plant density (10 and 20 plants m^{-2}) resulted in distinguishable romaine head formation, and plants visual quality worsened with higher plant density conditions (30 – 44 plants m^{-2}). Smith et al. (2011) reported that planting romaine lettuce with 0.05 – 0.075

m spacing and thinning them to 0.25 – 0.35 m (6.7 – 10 plants m^{-2}) resulted in full-sized heads after 65-80 days. Reduction in planting density improved the plant vegetative growth which produced maximum fresh mass of the plant (Hasan et al., 2017). Peet and Willits (1982) reported that reduced planting density produced larger plants with 61 % higher yield than the high-density plants. Makhadmeh et al. (2017) reported that increasing the planting density increased the competition among the plants thereby resulting in reduction in plant size. Silva et al. (2000) studied the effect of planting density on lettuce plant morphology. Tighter spacing condition resulted in increased competition for light thereby promoting the plant height leading to elongation (Figure 4). Meanwhile, reduction in planting density reduced the competition for light thereby reducing the stem growth which increased the diameter of the plant. Robinson (1970) conducted a study on effect of planting density on lettuce (cv. Climax) head formation and found that plant spacing of 35.56 cm × 35.56 cm (14 × 14 inch) spacing produced highest number of heads per carton $acre^{-1}$. Meanwhile, reduced plant spacing of 25.4 cm × 25.4 cm (10 × 10 inch) produced highest plant yield per unit area (45 tons $acre^{-1}$). The increases of spacing showed increasing trend in fresh mass of plant. In case of wider spacing, plants receive enough light and nutrients which leads to attain individual maximum fresh mass of plant (Boroujerdnia and Ansari, 2007; Rincon et al., 1998; Tittonell et al., 2003). Hence, optimum plant spacing ensured maximum vegetative growth that ensured highest fresh mass plant (Hasan et al., 2017). Therefore, for lettuce to produce distinguishable marketable heads an optimum planting density is needed.

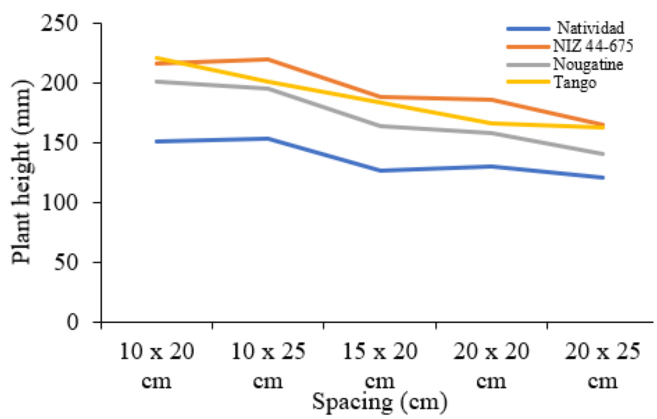

Figure 2.3 Plant height response of different lettuce cultivars in various plant spacings (Maboko and Du Plooy, 2009).

2.11 Planting density effect on hormones

Plant hormones play a crucial role in regulating plant growth in response to both internal and external signals (Vysotskaya et al., 2017). The presence of neighboring plants increases the sensitivity of plants which encourages plant elongation thereby increasing the level of abscission reducing the plant productivity. This promotes yield penalty in the plants (Heindl and Brun, 1983; Rousseaux et al., 1997). Increasing the planting density reduces the growth due to plant competition which increases the accumulation of abscisic acid (ABA) in the plant apex of the competing plants. The increase in ABA concentration in the shoots of competing plants at higher planting densities is likely to hinder the accumulation of biomass as ABA is well known as the plant growth inhibitor (Planes et al., 2015). Vysotskaya et al. (2018) reported that increased planting density of lettuce led to a significant two-fold rise in the shoot-to-root ABA ratio in grouped lettuce plants compared to individual plants. This relationship indicates that closer plant spacing promotes the transport of ABA from roots to shoots, which in turn may contribute to the observed growth inhibition. This increased concentration of ABA in high density lettuce planting thereby led to reduced shoot mass production. Planting density also influences the cytokinin levels in the plants. Arkhipova et al. (2015) reported a reduction in cytokinin concentration at tighter planting conditions. Vysotskaya et al. (2018) reported the absence of involvement of cytokinin in the short-term response studied with increased planting density of lettuce. It was shown previously that cytokinin can modify only the long-term growth response to the presence of neighbors (Arkhipova et al., 2015). Vysotskaya et al. (2017) studied of the effects of planting density on biomass accumulation and hormone levels and reported a change in concentrations of ABA, auxin, and cytokinin’s in the growth response of lettuce. This study resulted the presence of accumulated ABA in shoots and decreased root auxin levels which were likely contributing factors to the inhibition of shoot and root growth in plants grown at higher planting densities. Although increased planting density led to reduced shoot biomass accumulation, it did not impact leaf area, indicating that leaf cell extension in competing plants was maintained by auxin present in the shoots. Auxins are well-known for their role in promoting cell extension in coleoptile and hypocotyl cells. Therefore, planting density influences the plant growth hormones which can affect the plant growth and development. Optimal spacing between plants is crucial for regulating plant hormones, which can impact plant growth and yield.

2.12 Conclusion

Several studies have correlated the effect of planting density on biomass production. Planting area in a controlled environment is crucial as it has limited space within which cultivation is carried out. The energy utilization in CEA increases per unit area. Therefore, there is a need to utilize the available space efficiently and effectively to optimize plant growth and development to attain maximum yield. By identifying the optimum planting area, the resource use efficiency and profit of the CEA facility can be improved. Planting density to determine the yield plateau of romaine lettuce in a controlled environment hydroponic system has still not been identified. Moreover, we studied the effect of different planting patterns on romaine lettuce growth and yield. The effect of internodal elongation and plant height were taken into consideration to identify the spacing in which plant lodging started. This was measured to identify at which spacing the plant etiolation stopped and the head formation started. As romaine lettuce is well known for its head and heart development the head and heart diameter were measured in different spacing conditions to identify which spacing provided a better romaine head.

Chapter 3: Assessing the effect of planting density on romaine lettuce growth and quality in a controlled hydroponic environment.

Abstract

Planting density is a significant factor that influences plant growth and development. Romaine lettuce (*Lactuca sativa* var. longifolia) is a widely consumed leafy green in North America. The planting density (number of plants per unit area) determines the head and heart development in a controlled environment plant factory. The optimum plant spacing and planting pattern for romaine lettuce in a controlled environment is still unknown. This study aimed to examine the impacts of different planting densities (388 (5.0 cm), 172 (7.6 cm), 97 (10.1 cm), 62 (12.7 cm), 43 (15.2 cm), and 32 (17.7 cm) plants·m^{-2}) using both staggered and row planting patterns on biomass production, plant growth attributes, and head and heart quality of romaine lettuce (Valley Heart) grown in an ebb and flow hydroponic system in a greenhouse. Increasing the plant spacing significantly increased the fresh and dry mass of romaine lettuce producing a yield plateau for the dry mass (8.3 g) at 17.2 cm. Staggering did not significantly impact head and heart development, with the head and heart formation observed from a planting spacing of 15.2 cm. Increased internodal elongation promoted plant height and internodal elongation, causing lodging in low spacing conditions (5.0 cm and 7.6 cm) thereby reducing the chances of head and heart development. Our results demonstrate that the optimal spacing and planting patterns required when selecting different plant densities is an effective way to promote head formation while reducing internodal elongation in low plant spacing conditions. This study demonstrates the significance of the planting density and planting patterns that may influence crop yield and morphology in controlled environment agriculture.

Keywords: Controlled environment agriculture, greenhouse, hydroponics, lettuce, plant density, staggered.

3.1 Introduction

Lettuce is a widely consumed leafy vegetable grown under field conditions and in controlled environments. This annual plant is self-pollinating and features a deep taproot with horizontal lateral roots close to the surface. According to Ryder and Whitaker (1976), lettuce originated in Egypt and then spread to Western Europe, where a distinct type of lettuce, characterized by a head, emerged. This variety eventually made its way to America and became one of the world's largest cultivated vegetables. A phylogenetic study conducted by Yang et al. (2007) unveiled the genotypic similarities and phylogenetic tree of lettuce. Romaine and leafy lettuces likely developed from the cross-pollination of the crisp head type. Cos, or romaine lettuce, is a distinct variety with its tall, upright structure, elongated crispy leaves, prominent midvein, and loaf-shaped head that forms after the rosette stage; it can weigh up to 750 grams (Mou, 2008).

Romaine lettuce typically develops narrow oval leaves tightly wrapped around to form a soft and compact structure (Ryder, 1979). Several factors influence head formation, including leaf size, petiole length, stem elongation, and leaf production rate. Optimal head formation is promoted by larger and broader leaves, shorter petioles, slow stem elongation, and reduced internodal elongation (Wien, 1997). These factors collectively form a head critical for producing high-quality lettuce. Lettuce may fail to form heads due to various factors, out of which bolting is the most common. The process of bolting, which entails stem elongation and the formation of flowers (Chen et al., 2019), is primarily driven by two distinct but interrelated mechanisms: differentiation of the inflorescence meristem and division of the intercalary meristem. The former leads to the emergence of the floral meristem, which subsequently gives rise to diverse floral structures. The latter enables the rapid extension of the stem by supplying additional cells from the intercalary meristem (Alvarez-Buylla et al., 2010), thus interrupting head formation, promoting stem elongation and leaf twisting (Nothmann, 1977). Lower than optimal temperature slows growth, and canopy extension is faster under higher temperature, leading to an increased rate of light interception resulting in bolting, bitterness, tip burn, and poor heading (Decoteau, 2000). Under high-density conditions, the competition for light intensifies among the plants, thereby affecting the canopy structure and leading to lodging (Shan et al., 2022).

The romaine lettuce industry is a substantial part of North America's economy due to the widespread consumption of fresh salads containing lettuce in the region (Bayley, 2020; Lu et al., 2022). In 2021, Canada imported around 267,000 metric tonnes of lettuce, which amounted to

CAD 559.2 million (Agriculture and Agri-food Canada, 2022). Out of this, 524.4 million CAD was spent on importing approximately 253,000 metric tonnes of lettuce from the United States, where the Salinas Valley is a significant lettuce-growing region (Wisler and Duffus, 2000). However, this region has experienced major disease outbreaks, including *E. coli* O157:H7 contamination, which caused disruptions in the supply of romaine lettuce in 2019 (Public Health Agency of Canada, 2022). This outbreak led to at least seven deaths in North America, and many people were sickened or hospitalized. Between 2010 and 2019, Canada issued 16 recalls linked to *E. coli* outbreaks in romaine lettuce. Prior to this, impatiens necrotic spot virus (INSV), a thrips-transmitted pathogen of lettuce rapidly emerged as a serious threat to lettuce production in the Salinas Valley of Monterey County (Kuo et al., 2014). As per the estimates of the Grower-Shipper Association of Central California, INSV caused a loss of revenue of $100 million to lettuce producers in 2020 (California Farm Bureau Federation, 2022). In 2022, California experienced dry weather conditions and inadequate rainfall, which, along with the incidence of INSV infection, led to the significant loss of romaine and iceberg lettuce crops in Canada (Dawson, 2022). This resulted in a shortage of lettuce supply, leading to a surge in lettuce prices. Therefore, outdoor cultivation of romaine lettuce is continuously threatened by pest, weather and disease outbreaks which causes a disruption in the supply chain management. Controlled environment agriculture has the potential to improve the yield by promoting uniform plant growth and development when compared to field conditions (Ahmed et al., 2020b). Lettuce has proven to be economically viable crop for operators of plant factories with electrical lighting (PFEL) who achieve a positive revenue-cost performance in its production (Zhuang et al., 2022). The implementation of efficient year-round indoor lettuce production in Canada holds potential for mitigating the country's dependence on imported produce. Thereby, the adoption of controlled environment agriculture can lead to a significant improvement in food safety and biosecurity (Benke and Tomkins, 2017).

Indoor farming is an environmentally sustainable agriculture method that conserves water, fertilizer, and land resources while preventing the need for pesticides (Van Delden et al., 2021). Indoor farming practice such as the vertical farming allows stacked production thereby increasing the production of plants per unit area (Beacham et al., 2019). Compared to conventional farming practices, vertical farming produces higher yield per unit area (Despommier, 2009). This system utilizes high-density planting techniques to increase crop productivity per unit area. The spatial distribution of plants in a crop community is an essential determinant of yield (Egli, 1988). The relationship between plant density and crop yield is typically characterized by a parabolic curve, whereby an optimal level of plant density is associated with maximum yields. Deviations from this optimal plant density, either too high or too low, can reduce crop yields in various crops (Ciampitti and Vyn, 2011; Hiltbrunner et al., 2007; Lin et al., 2009). The microenvironment of the crop canopy, which encompasses variables such as light, temperature, and relative humidity, is of paramount importance for crop growth and development (Yang et al., 2014). The influence of plant density on the canopy microenvironment is substantial and can significantly affect the growth and development of crops (Yang et al., 2014). Modifying the density of plant populations can lead to changes in the structure of the canopy and root system architecture, thereby impacting the way in which plants absorb and utilize radiation, water, and nutrients (Zhang et al., 2021).

To ensure maximum yield during reproductive growth in plant communities, it is crucial to have sufficient leaf area (Johnson et al., 1992; Shibles, 1965; Tanner and Hume, 1978). Increasing planting density is a globally recognized agronomic practice for enhancing the plant yield and ensuring resource use efficiency. This method promotes rapid canopy closure and increases the leaf area index, which enhances the interception of photosynthetically active radiation (IPAR) and increases plant biomass production, leading to higher yields (Du et al., 2021; Hernandez et al., 2020; Teixeira et al., 2014; Zhang et al., 2021). According to Bégué (1993) intercepted photosynthetic active radiation is defined as the radiation absorbed by vegetation within a canopy, and it is calculated as the difference between the incoming radiation at the top of the canopy and the radiation transmitted downwards and reaching the bottom of the canopy. High density planting can improve the rate of IPAR, but it can also escalate the competition between the plants for crucial resources such as water, light and nutrients (Ciampitti and Vyn, 2011; Rossini et al., 2011). However, competition for light, water, and nutrients among plants increases with greater planting density, reducing crop productivity and resource use efficiency by decreasing light interception and photo assimilate production (Du et al., 2021; Teixeira et al., 2014; Zhang et al., 2019). Equidistant spacing between plants is recommended to minimize interplant competition and maximize yield, as this is the most effective approach (Pendleton and Hartwig, 1973; Wiggans, 1939).

The primary objective of the study was to determine optimal spacing conditions for romaine lettuce grown in controlled environment by investigating the effect of staggered and non-staggered spacing configurations on plant growth and development. It was hypothesized that staggering the plant spacing would benefit the romaine lettuce in terms of biomass yield, as well as head and heart development. The relationship between plant height and internodal elongation on head and heart formation of romaine lettuce was further explored; it was hypothesized that increased internodal elongation can cause bolting. Findings reported here may prove valuable for plant production in a controlled environment leafy green production. Identifying the optimum plant yield facilitates efficient usage of resources thereby improving the yield per unit area.

3.2 Materials and methods

3.2.1 Experimental design and site description

This experiment was conducted as a randomized complete block design, having a total of 12 treatments which includes both staggered and non-staggered spacing with three replicates. Six different planting densities were labelled from A to F (i.e., A – 5.0 cm, B – 7.6 cm, C – 10.1 cm, D – 12.7 cm, E – 15.2 cm, F – 17.7 cm) A_1- non-staggered and A_2- staggered (Figure 3.2). The treatments were randomly distributed in the bench at the greenhouse. These 12 treatments were replicated over three temporal cycles with each temporal cycle acting as a block. The treatments were randomized within each block. This study was conducted from November 2021 to January 2023 in McGill University's Macdonald campus (Montreal, QC, Canada, N 45° 24' 28.5768", W 73° 56' 19.7916") research greenhouse bay #07. The greenhouse is equipped with a high-pressure sodium (HPS) lighting system (P.L. Light systems, Ontario, Canada) with a broad spectrum and controlled intensity (Figure 3.1). Air temperature and the relative humidity were monitored in the greenhouse using an aspirator box (Priva B.V., De Lier, Netherlands). Horizontal air flow (HAF) fans (Schaefer, North Carolina, US) were used to maintain uniform air flow. Monthly average canopy temperature, relative humidity, light intensity, water temperature, and electrical conductivity (EC) of the hydroponic solution were measured (Table 3.1) using the raspberry pi system (section 3.2.3).

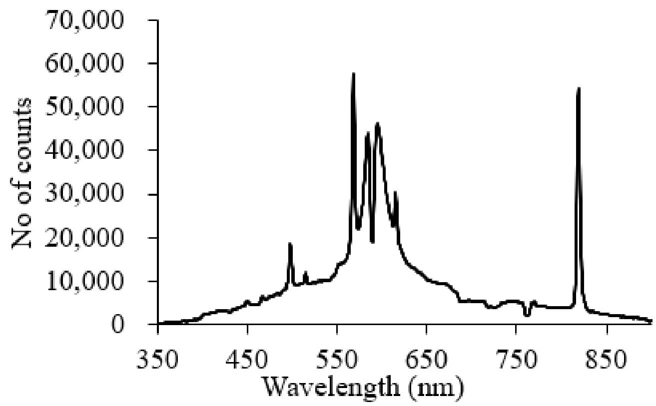

Figure 3.1 High pressure sodium (HPS) with broad wavelength used in the greenhouse measured using the spectroradiometer.

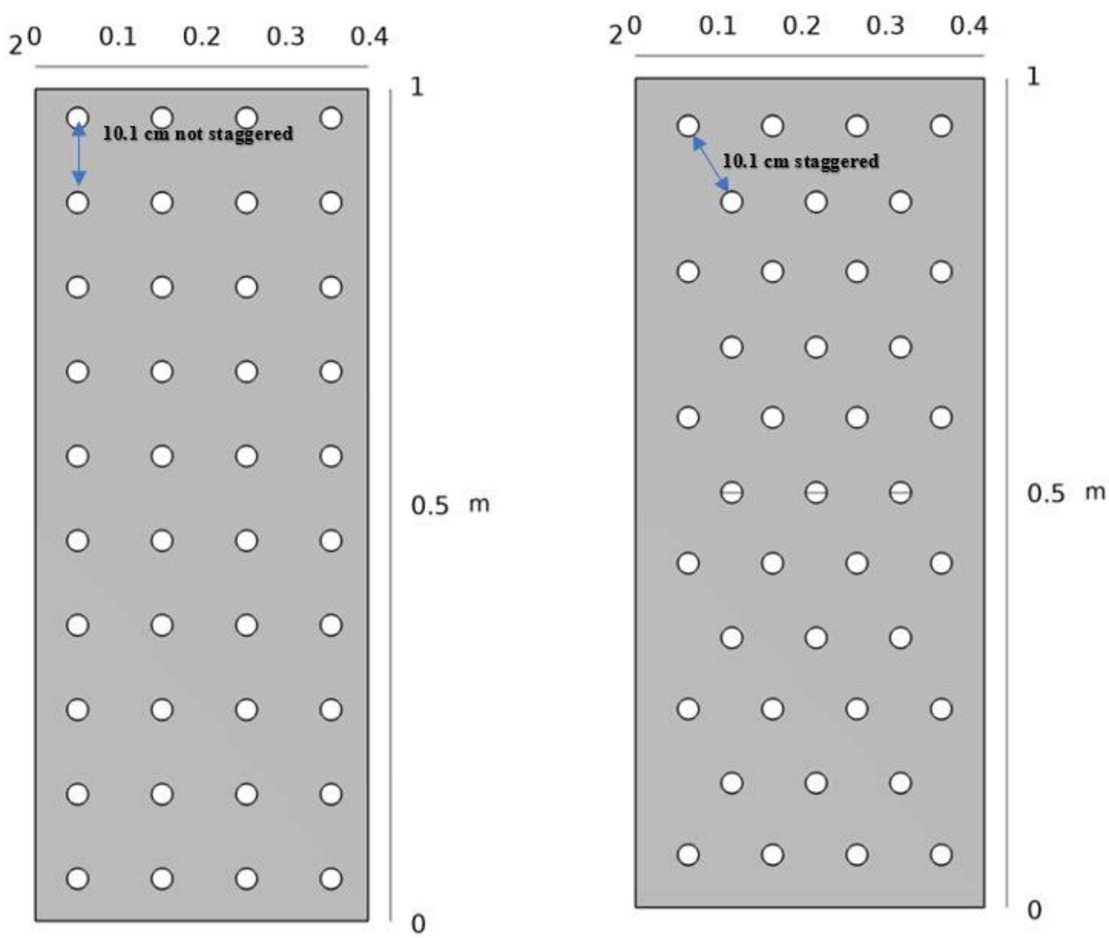

Figure 3.2 Schematic representation of density spacing where rockwool cubes with lettuce seeds were spaced 5.0 cm, 7.6 cm, 10.1 cm, 12.7 cm, 15.2 cm, and 17.7 cm apart in non-staggered (left) and staggered (right) configuration.

Table 3.1 Experimental environmental conditions for romaine lettuce germinated in the walk-in growth chamber (days 0–21) and grown in the research greenhouse (days 21–70)

Abiotic Factors	Growth Chamber (Days 0–21)	Greenhouse (Days 21–70)
Type of lighting	Fluorescent bulbs	Sunlight & HPS (High-Pressure Lighting)
Light spectrum	400–500 nm	400–700 nm
Photoperiod (Day/Night) (h)	16/8	16/8
Photosynthetic photon flux density (PPFD) (μmol m^{-2} s)	100	200
Temperature (Day/Night) (°C)	20/16	20/16
Relative humidity (%)	60±5	55±5
Water temperature (°C)	22	22
Hydroponic solution	Full strength Hoagland's solution 14 days after seeding	Full strength Hoagland's solution from the day 21 after seeding
pH	6.0 ± 0.5	6.0 ± 0.5
Electrical conductivity (μS)	1000±500	1000±500

3.2.2 Planting material

Lettuce seeds (cv. Valley heart) (Stokes seed Ltd., Thorold, ON, Canada) were double seeded in rockwool cubes (Grodan, Milton, ON, Canada) to increase germination rate and placed in a walk-in growth chamber (Conviron, BDW80, Manitoba, Canada). The germinated seedlings were thinned out to maintain one seedling per rockwool cube. The seedlings were irrigated solely with water for the first two weeks. After 14 days, the seedlings were provided full-strength Hoagland's solution (Hoagland and Arnon, 1950). After 21 days of seeding, the seedlings were transferred from the walk-in growth chamber to the ebb-and-flow hydroponic tray (Qualiplast, Quebec, Canada) in the research greenhouse, where full-strength Hoagland's solution was used as the nutrient medium as of the first day of the transplant. The nutrient solution was stored in a plastic bin (Rubbermaid, Atlanta, Georgia, United States), and pumped to the ebb and flow system with a submersible water pump (Ecoplus, Austin, Texas, United States). The nutrient solution was changed every two weeks to avoid salt accumulation which can increase electrical conductivity (EC) and total dissolved solids (TDS). Water samples from the hydroponic system were collected monthly once and sent for analysis (A&L Canada Laboratories, London, Ontario, Canada).

3.2.3 Environmental data monitoring

Raspberry Pi 4 Model B (Trxcom, Shenzhen, China) was used as an automated agricultural system to monitor environmental parameters in the greenhouse. To measure temperature and relative humidity, a SHT30 sensor (Sincere & Promise, Shenzhen, China) and a I2C communication protocol was used with a temperature range of 0 to 65 °C and a humidity range of 0 to 100 %. To monitor light intensity, the VEML7700 (Adafruit, New York, US) was used with ambient light digital 16-bit resolution and a light range of 0 to 120000 lux, where the values were converted to µmol m^{-2} s^{-1}. Root temperatures were monitored with a one wire communication sensor DS18B20 (Maxim, California, US) that ranged from 55 °C to 125 °C. The pH (0 to 14) and the EC (0 to 3999 µS cm^{-1}) of the hydroponic solution were measured by using HI98129 (Hanna, Rhode Island, US). The sensors were connected to the Raspberry Pi system and environmental data was collected every 3 minutes.

3.2.4 Experimental data collection

Romaine lettuce was harvested 70 days after being seeded, and eight representative samples were randomly selected from each tray for phenotypic characterization. Fresh mass, dry mass, plant height, internodal distance, head diameter, and heart diameter were measured. Fresh and dry mass was determined by weighing the samples before and after drying at 65 °C for 72 h in an isotemp oven (Fisher, New Hampshire, US) until a stable mass was attained. Plant height was measured by using a ruler (Eboot, Massachusetts, US) from the stem portion of the lettuce (i.e., collar) to the plant's apex. Internodal distance was determined to assess internodal elongation. To measure internodal elongation in plants, the distance between the 4^{th} and 5^{th} internodes was measured from the apical meristem as it is the portion where the stem elongation begins. The head diameter of the plant was measured using a vernier caliper (Neotech, Kowloon, Hong Kong) by placing the plant's head between the caliper jaws. The jaws of the caliper were used to hold the head in place without damaging it. Heart diameter was measured after the head leaves were manually removed. As similar to head measurement the heart diameter was measured using the caliper.

3.2.5 Statistical analysis

Data was analyzed using randomized complete block design model SAS 9.4 software (SAS Institute, Cary, NC). Statistical analyses were conducted to determine the effect of block (temporal cycle), independent treatment effects (plant spacing and staggered/non-staggered), interactions between treatments, and the treatment- block interaction effect on the plant’s responses such as fresh mass, dry mass, plant height, internodal distance, head and heart diameter, head, and heart leaves. Means and standard deviations (SD) of the measured values were determined using analysis of variance (ANOVA). Fisher’s Protected LSD test at the 5 % level of significance was used to compare treatment means.

3.3 Results

Table 3.2 Effect of plant spacing on romaine lettuce yield. Means in a column followed by the same letter are not significantly different ($p > 0.05$), using Fischer's LSD test.

Plant spacing	No of observations	Fresh mass (g)	Dry mass (g)	Plant height (cm)	Internodal distance (cm)	Head dimension (cm)	Heart dimension (cm)	Head leaves (#)	Heart leaves (#)
A_1	8	44.4 ± 4.9^{a}	2.17 ± 0.75^{a}	46.1 ± 2.2^{ab}	3.4 ± 0.2^{a}	0 ± 0.3^{a}	0 ± 0.2^{a}	0 ± 0.4^{a}	0 ± 0.3^{a}
A_2	8	40.7 ± 4.9^{a}	2.81 ± 0.75^{ab}	48.7 ± 2.2b	3.0 ± 0.2^{ab}	0 ± 0.3^{a}	0 ± 0.2^{a}	0 ± 0.4^{a}	0 ± 0.3^{a}
B_1	8	77.7 ± 4.9^{b}	3.86 ± 0.75^{abc}	43.5 ± 2.2^{abc}	2.4 ± 0.2^{bc}	0 ± 0.3^{a}	0 ± 0.2^{a}	0 ± 0.4^{a}	0 ± 0.3^{a}
B_2	8	$64.2\ 0 \pm 4.9^{b}$	4.78 ± 0.75^{bd}	41.1 ± 2.2^{ad}	1.9 ± 0.2^{cd}	0 ± 0.3^{a}	0 ± 0.2^{a}	0 ± 0.4^{a}	0 ± 0.3^{a}
C_1	8	111.1 ± 4.9	5.79 ± 0.75^{cde}	37.3 ± 2.2^{cd}	1.9 ± 0.2^{cd}	0 ± 0.3^{a}	0 ± 0.2^{a}	0 ± 0.4^{a}	0 ± 0.3^{a}
C_2	8	93.6 ± 4.9	3.97 ± 0.75^{ad}	39.3 ± 2.2^{cd}	1.5 ± 0.2^{de}	0 ± 0.3^{a}	0 ± 0.2^{a}	0 ± 0.4^{a}	0 ± 0.3^{a}
D_1	8	132.5 ± 4.9	5.70 ± 0.75^{cdf}	39.3 ± 2.2^{cd}	1.4 ± 0.2^{df}	0 ± 0.3^{a}	0 ± 0.2^{a}	0 ± 0.4^{a}	0 ± 0.3^{a}
D_2	8	154.7 ± 4.9	5.94 ± 0.75^{cdf}	37.2 ± 2.2^{cd}	1.2 ± 0.2^{efg}	0 ± 0.3^{a}	0 ± 0.2^{a}	0 ± 0.4^{a}	0 ± 0.3^{a}
E_1	8	190.3 ± 4.9^{c}	7.30 ± 0.75^{efg}	35.8 ± 2.2^{d}	0.9 ± 0.2^{fh}	10.7 ± 0.3^{b}	7.5 ± 0.2^{b}	10.1 ± 0.4^{b}	14.4 ± 0.3^{b}
E_2	8	204.3 ± 4.9^{cd}	7.47 ± 0.75^{efg}	35.7 ± 2.2^{d}	0.8 ± 0.2^{fh}	11.5 ± 0.3^{bc}	8.1 ± 0.2^{bc}	9.0 ± 0.4^{b}	14.7 ± 0.3^{b}
F_1	8	213.9 ± 4.9^{d}	8.34 ± 0.75^{g}	34.7 ± 2.2^{d}	0.6 ± 0.2^{gh}	11.8 ± 0.3^{cd}	8.6 ± 0.2^{c}	9.4 ± 0.4^{b}	15.0 ± 0.3^{b}
F_2	8	205.3 ± 4.9^{d}	8.3 ± 0.75^{g}	34.7 ± 2.2^{d}	0.5 ± 0.2^{h}	$11.5 \pm 0.3b^{d}$	8.5 ± 0.2^{c}	9.6 ± 0.4^{b}	15.0 ± 0.3^{b}

A_1, B_1, C_1, D_1, E_1, F_1 - 5.0 cm, 7.6 cm, 10.1 cm, 12.7 cm, 15.2 cm, 17.7 cm non-staggered respectively**

A_2, B_2, C_2, D_2, E_2, F_2 – 5.0 cm, 7.6 cm, 10.1 cm, 12.7 cm, 15.2 cm, 17.7 cm staggered respectively**

3.3.1 Optimum planting density for Valley Heart lettuce

The optimal planting density, defined as the number of plants per unit area, plays a crucial role in determining the yield per unit area of Valley Heart lettuce. In this experiment, Valley Heart lettuce plants were selected from all treatments, including those with varying available space in the corners and sides. By considering these plants with different spacing, it was possible to extrapolate the planting density and identify the optimum spacing for maximizing yield. Lower spacing condition such as 5.0 cm produced plants with lower mass meanwhile increasing the plant spacing improved the yield of Valley Heart, indicating that increasing the spacing between plants can enhance the fresh mass yield of Valley Heart lettuce under controlled environmental conditions. As the planting density continued to increase, the fresh mass eventually reached a plateau at a spacing of 20 cm attaining yield around 200 g, which further stabilized at a spacing of 25 cm yielding up to 230 g (Figure 3.3). Beyond this point, increasing the plant spacing did not produce any additional yield, with yield remained staggered at 230 g indicated that further space allocation was not advantageous for plant growth (Figure 3.3). Therefore, the identified yield plateau for Valley Heart lettuce corresponds to a planting density of 25 cm. Implementing this optimal spacing in controlled environments can result in higher and more economically beneficial yields. This finding highlights the significance of selecting the appropriate planting density to achieve maximum fresh mass production in Valley Heart lettuce.

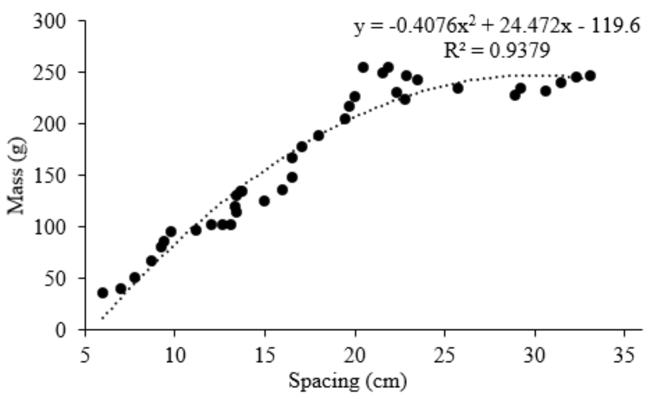

Figure 3.3 Yield density curve of Valley Heart grown in controlled environment hydroponic system.

3.3.2 Fresh and dry mass

The optimal spacing conditions for romaine lettuce grown in controlled environment and the effect of staggered and non-staggered spacing configurations on plant growth and development was studied in this experiment. Increasing plant spacing from 5.0 cm to 17.7 cm had a significant ($p < 0.05$) impact on both the fresh mass and dry mass yields (Table 3.2). Specifically, the fresh mass and dry mass yields increased by 128.8 % and 117.1 %, respectively (Table 3.2). The highest fresh mass yield of 213.9 g was observed in the 17.7 cm non-staggered spacing condition. This was followed by the 17.7 cm staggered spacing condition, which produced a yield of 205.1 g, and the 15.2 cm staggered plant spacing condition, which yielded 204.1 g (Figure 3.4A). Staggering the planting pattern across all spacing configurations did not yield a significant impact ($p > 0.05$) on the fresh mass when compared to their corresponding non-staggered spacing conditions (Table 3.2). Regarding the dry mass yield, the highest yield of 8.3 g was recorded in the 17.7 cm non-staggered spacing condition, followed by the 17.7 cm staggered and 15.2 cm staggered conditions, which yielded 8.3 g and 7.4 g, respectively (Figure 3.4B). Staggering the plant spacing in every other spacing configuration (5.0 cm, 7.6 cm, 15.2 cm, 17.7 cm) did not have a significant impact ($p > 0.05$) on dry mass when compared to its corresponding non-staggered spacing except 10.1 cm and 12.7 cm (Table 3.2). While staggering the planting pattern reduced the fresh yield by 8.1 %, 16.7 % 15.7 % and 4.1 % in 5.0 cm, 7.6 cm, 10.1 cm, and 17.7 cm spacings respectively but improved the yield in the 12.7 cm and 15.2 cm spacing conditions by 15.4 % and 7 % when compared to their corresponding non-staggered spacing conditions respectively (Table 3.2).

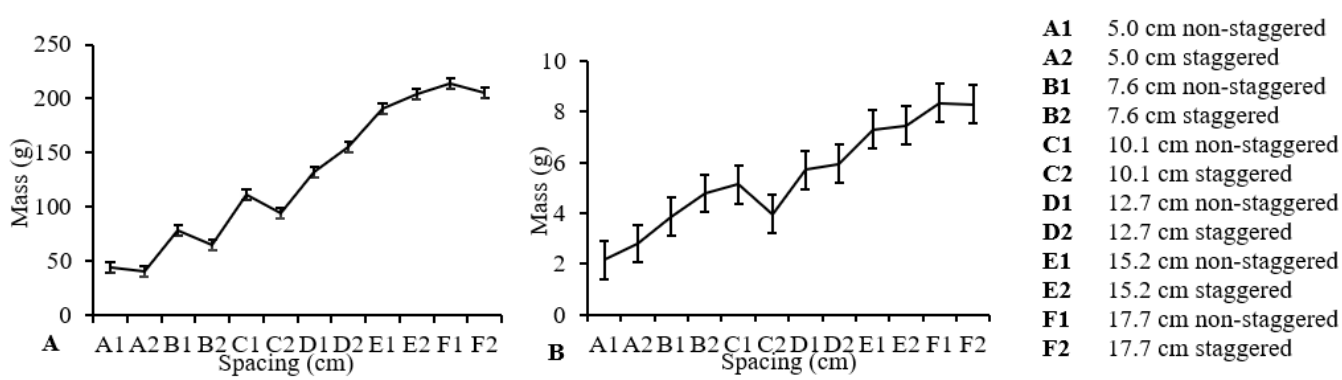

Figure 3.4 Fresh (A) and dry (B) yield of romaine lettuce in different spacing conditions.

3.3.3 Plant height and internodal elongation

Planting pattern and density had a significant effect ($p < 0.05$) on both plant height and internodal elongation. The shortest plant height of 34.7 cm was observed in 17.7 cm both staggered and non-staggered spacing conditions (Figure 3.5A). The highest plant height of 48 cm was recorded in the highest planting density of 5.0 cm staggered, with bolting and lodging observed. Reducing the planting density from 5.0 cm to 17.7 cm reduced both the plant height and internodal elongation by 28.1 % and 146.9 % (Table 3.2), respectively. Increase in plant spacing resulted in a reduction in plant height, particularly between the spacing of 5.0 cm and 12.7 cm. Both staggered and non-staggered planting configurations at 15.2 cm and 17.7 cm spacing exhibited a more stationary plant height, indicating a reduction in bolting and elongation. Staggered spacing did not have a significant effect ($p > 0.05$) on plant height in comparison to non-staggered spacing in all spacing conditions (Table 3.2). The highest internodal elongation of 3.4 cm was recorded with the 5.0 cm non-staggered condition, whereas the lowest elongation of 0.5 cm was observed with the 17.7 cm staggered spacing condition (Figure 3.5B). The application of a staggering planting pattern resulted in a reduced internodal elongation across all spacing conditions (Table 3.2). Wider spacing conditions 15.2 cm and 17.7 cm both staggered and non-staggered condition resulted in internodal elongation less than 1 cm (Table 3.2). Increasing the plant spacing form 5.0 cm to 17.7 cm significantly reduced the internodal elongation (Figure 3.5B). However, when comparing the specific staggered spacing condition to its corresponding non-staggered spacing condition, the observed effect was not statistically significant ($p > 0.05$) (Table 3.2).

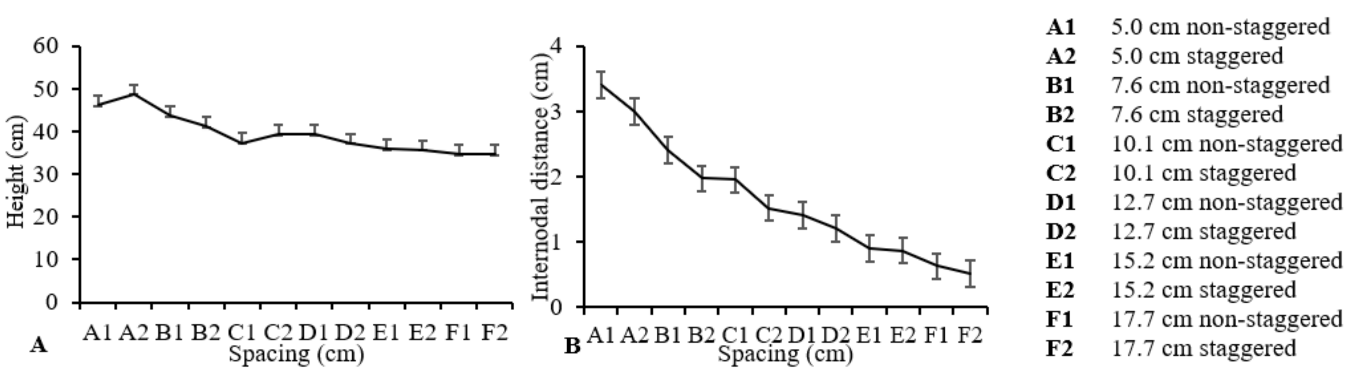

Figure 3.5 Plant height (A) and internodal elongation (B) of romaine lettuce in different spacing conditions.

3.3.4 Head and heart formation

In this experiment, the head and heart formation of Valley Heart was measured in different spacing conditions. The compact and tightly packed cluster of leaves, providing a firm structure to the lettuce is commonly known as the head. The heart refers to the central portion of the Valley Heart where these leaves originate. The head and heart formation were observed in the romaine lettuce when the plants were spaced 15.2 cm apart in a non-staggered planting configuration which improved with further increase in spacing (Figure 3.6). Lower spacing conditions (5.0 cm, 7.6 cm, 10.1cm and 12.7 cm) did not promote the formation of lettuce heads and hearts. Until this point, loose lettuce leaves were produced without significant signs of head and heart formation. When staggered planting pattern was implemented under these spacing conditions, it did not result in the development of heads and hearts. The largest head and heart diameters of 11.8 cm and 8.6 cm were observed with a 17.7 cm non-staggered spacing condition (Figure 3.6). Increased plant spacing such as 15.2 and 17.7cm with both staggered and non-staggered configuration had a significant effect ($p < 0.05$) on head and heart formation over other spacing conditions (5.0 cm, 7.6 cm, 10.1 cm, 12.7 cm) (Table 3.2). Staggering did not have a significant effect ($p > 0.05$) on head and heart formation in different spacing condition (Table 3.2). No tipburn was observed in any romaine lettuce throughout the experiment.

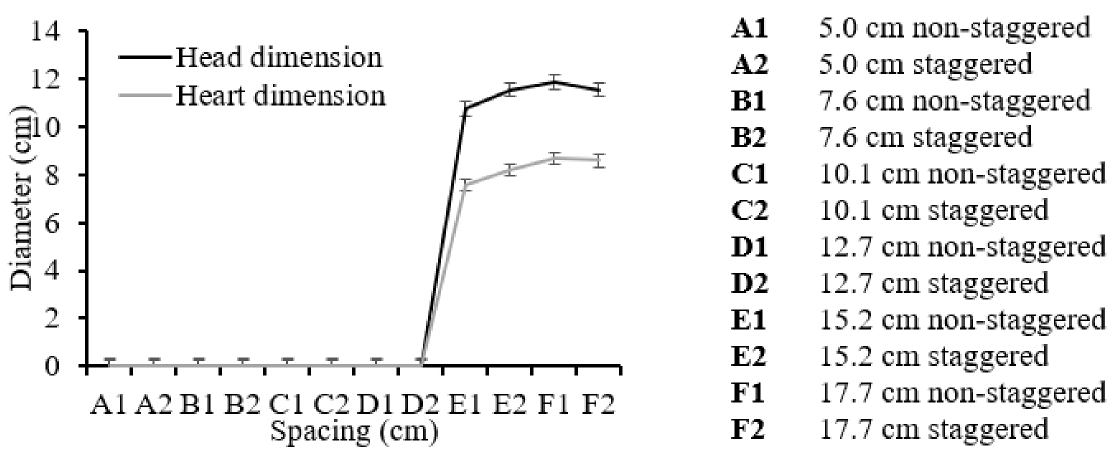

Figure 3.6 Head and heart diameter of romaine lettuce in varying spacing condition.

3.3.5 Head and heart leaves

In Valley Heart the leaves which curved away from the center of the plants were identified as the head leaves. The point where the curvature of the leaves changed from curving away from the center of the plant to curving towards the center of the plant was identified as the starting point of the heart-forming leaves. When lettuce plants were spaced from 15.2 to 17.7 cm apart, the number of head and heart leaves increased, indicating a transition from loose leaf to head lettuce (Figure 3.7). As of 15.2 cm head and heart formation was observed, head and heart leaves were similarly observed with this spacing. When lettuce plants were spaced 15.2 to 17.7 cm apart, the number of head and heart leaves increased alongside. Reduced spacing conditions such as 5.0 cm, 7.6 cm, 10.1 cm, and 12.7 cm) did not have a significant effect ($p > 0.05$) on head and heart leaf formation. Staggering the plant spacing did not have a significant effect ($p > 0.05$) on head and heart leaves (Table 3.2). The highest number of head and heart leaves (11.9 and 15.1, respectively) were observed with 17.7 cm spacing in non-staggered configuration (Table 3.2). Staggered 17.7 cm plant spacing had a reduced number of head and heart leaves, when compared to the 17.7 cm non-staggered configuration (Figure 3.7).

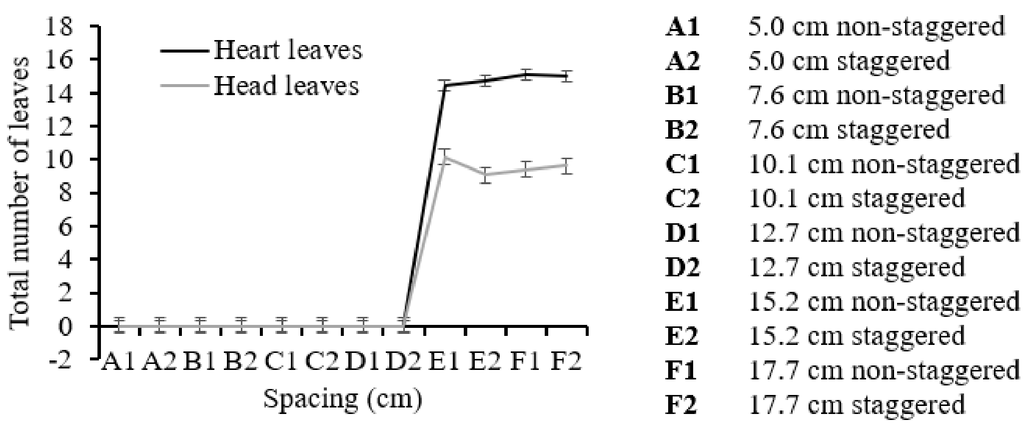

Figure 3.7 Head and heart leaves of romaine lettuce in varying spacing condition.

3.4 Discussion

Findings from this study suggest that plant spacing and planting pattern play a critical role in determining the yield and morphological characteristics of romaine lettuce in a controlled environment hydroponic system. This supports previous research investigating planting density where yield per unit area reportedly increases within a particular density range for each pattern without causing interplant competition for light (Duncan, 1986). Square and triangular patterns are considered more efficient in utilizing surrounding areas by extending their branches in all directions, making the most of available resources (Miura and Gemma, 1986), more so than equidistantly spaced plants arranged in a rectangular manner (Harper, 1983; Wiggans, 1939). Therefore, planting pattern is a critical factor in maximizing crop yield by efficiently utilizing resources. Data collected in this study showed that increasing plant spacing significantly ($p < 0.05$) increases fresh and dry mass of romaine lettuce (Table 3.2). Non-staggered planting had a positive impact on fresh mass, particularly when lettuce plants were spaced 5.0 cm, 7.6 cm, and 10.1 cm apart, staggered planting with the same spacing resulted in lower yields. When plants were spaced 12.7 cm and 15.2 cm apart, non-staggered planting had reduced yields compared to the yields with the staggered planting (Figure 3.4A). Maximum fresh mass yield was achieved with plants spaced 17.7 cm apart in a non-staggered configuration (Figure 3.4A), which is consistent with the findings of a study by Bayley (2020) that reported the highest yield of hydroponic lettuce production with a plant spacing of 18 cm. For romaine lettuce grown in the field, a plant spacing of 30 cm has been recommended to obtain maximum yield per plant (Chu et al., 2016).

This study further revealed that staggering had a significant impact ($p < 0.05$) on dry yield at lower spacings, such as when plants were spaced 5.0 cm and 7.6 cm apart, while it had no significant influence on other plant spacings. Dry yield increased as plant spacing increased, with the maximum yield obtained at 17.7 cm for both staggered and non-staggered spacing (Figure 3.4B). When plant spacing was set at 17.7 cm, the dry yield of romaine lettuce became constant for both staggered and non-staggered configurations, indicating the same yield plateau for dry yield reported previously for 18 cm spacing (Bayley, 2020). This suggests that plant spacing beyond this point will not be beneficial. Furthermore, the results indicated that plant spacing affected the morphological characteristics of romaine lettuce, such as plant height, internodal elongation, and head and heart formation. The lettuce plants started bolting and lodging in low plant spacing conditions (i.e., 5.0 cm and 7.6 cm apart), indicating that competition for light intensified among the plants, increasing leaf overlapping and promoting an increase in plant height and internodal elongation (Figure 3.5A & 3.5B). In this study, we found that staggering had a significant impact ($p < 0.05$) on plant height in low plant spacing conditions, reducing internodal elongation. However, increased plant spacing with staggering did not have a significant impact (p

> 0.05) on internodal elongation, and non-staggered planting conditions had increased internodal elongation than the staggered planting pattern (Table 3.2). Our study investigated the effect of spacing distances (5.0 cm, 7.6 cm, 10.1 cm, 12.7 cm, 15.2 cm, and 17.7 cm) on lettuce's visual quality. Reduced spacing conditions resulted in a decline in visual quality, supporting the notion of poorer visual characteristics under tighter spacing. Better visual quality was observed in spacing conditions of 15.2 cm and 17.7 cm. This finding aligns with a study conducted by Bayley (2020) that assigned visual quality scores to lettuce at different densities. This study also concluded that lower spacing conditions produced lettuce with poor visual score and quality. This emphasizes the significance of appropriate spacing for maintaining optimal visual quality in lettuce cultivation.

3.5 Conclusion

In conclusion, this study highlights the importance of plant spacing and planting patterns for maximizing yield and morphological characteristics of romaine lettuce in a controlled environment hydroponic system. A yield plateau for dry yield in Valley Heart lettuce was identified at a plant spacing of 17.7 cm. Highest fresh yield of romaine lettuce in a controlled environment hydroponic system was observed in 17.7 cm spacing condition. Plant spacing beyond 25 cm was found to be non- profitable indicating that Valley Heart has attained its yield plateau around this spacing. Reduced plant spacing increased bolting and internodal elongation in Valley Heart resulting in poor visual quality thereby reducing the marketable quality. Wider spacing conditions such as 15.2 cm and 17.7 cm reduced plant elongation and promoted both head and heart development. Although staggering increased the fresh and dry yield of Valley Heart in various spacing conditions it did not have a significant effect ($p > 0.05$) when compared to the corresponding non-staggered spacing configuration. Staggering the plant spacing reduced the plant height and internodal elongation indicating that altering the planting pattern can reduce the effect of bolting. Highest head and heart diameter was observed in plant spacing of 17.7 cm non-staggered spacing configuration. To achieve a marketable Valley Heart head a plant spacing of 17.7 cm is recommended. Findings may provide insight into the optimal spacing and planting patterns required when selecting different plant densities, and staggering can be an effective way to promote head formation while reducing internodal elongation in low plant spacing conditions. Further research is required to elucidate the significance of these effects and to explore other factors that may influence crop yield and morphology in controlled environment agriculture.

Increasing planting density resulted in worsening visual quality, however not significantly so, indicating a possible increase in morphological defects, and an area where further research is required.

Chapter 4. Effect of plant spacing and spatial arrangement on different cultivars of romaine lettuce in a controlled hydroponic environment system

Abstract

Planting density, or the number of plants per unit area, is an important factor in determining the production capacity of a controlled environment plant factory. The aim of this study was to investigate plant competition by examining how plant growth and productivity are affected by plant density, focusing on two romaine lettuce cultivars (*Lactuca sativa* var. longifolium cvs. Valley Heart and Breen). To compare the performance of these cultivars, four different spacing conditions were implemented in an ebb and flow hydroponic system in the research greenhouse at the McGill's Macdonald campus. Both square and rectangular spacing conditions were utilized to assess lettuce head and heart formation. Valley Heart had a higher yield in lower spacing conditions than Breen. The formation of heads and hearts varied among cultivars in different spacing conditions. Breen exhibited the potential to form heads and hearts in lower density conditions, while Valley Heart demonstrated a tendency to bolt under these same conditions. These findings contribute to a better understanding of different cultivars' responses to planting density in terms of productivity, providing valuable insight into optimizing lettuce cultivation in hydroponic systems.

Keywords: Planting density, Breen, Valley heart, hydroponics, bolting, head formation.

4.1 Introduction

Lettuce is a well-known leafy green distributed worldwide, originating from the Mediterranean region and belonging to the family Asteraceae (Funk et al., 2005). Lettuce is famous worldwide for its usage and consumption as a salad. Lettuce, including the romaine (cos) type (cv. longifolia), are species with ideal characteristics for hydroponic culture. These leafy vegetables have a relatively short growing period and a low requirement for nutritional medium (Cahn and Johnson, 2017; Lei and Engeseth, 2021). There are two types of romaine lettuce based on plant height: regular lettuce or maxi, which can grow up to 40 cm, and baby lettuce, which can grow up to 30 cm in size. This classification was extended to include a subcategory with regards to the size (height) reached by the plants, known as the mini (also known as a baby), with an average height of up to 20 cm (Grzegorzewska et al., 2023; López et al., 2014). Romaine lettuce may be further classified by head structure and size, such as normal romaine with long and unbroken leaves, a broad midrib, and an open head ranging up to 20–26 cm in length. Mini romaine is similar in morphology, but the leaf length ranges between 18–20 cm long (López et al., 2014). The mini and midi types are the most popular for hydroponic cultivation (Grzegorzewska et al., 2023).

In a controlled environment hydroponics system, biomass production is directly correlated to the number of plants per unit area. Bugbee and Salisbury (1988) identified three essential physiological factors: the plants' capacity to capture radiation, their quantum yield or efficiency in converting this radiation into energy, and their efficiency in respiration or carbon use which predominantly determine the plant biomass production capacity. During the early stages of crop growth and development, plant density determines the leaf area for maximum utilization of solar radiation. Higher planting density influences plant architecture, which changes the leaf size and shapes, promoting vertical growth of leaves and facilitating higher solar radiation interception (Brown, 1968; Newton and Blackman, 1970; Pearce et al., 1967). Planting density is crucial in determining plant yield and architecture (Takahashi and Cardoso, 2014). there is a threshold level, beyond which an increase in planting density does not improve the plant yield (Janick, 1968). Increased planting density increases the competition for resources between the plants, reducing plant growth and affecting the yield attributes (Schroeder and Janos, 2005). Higher planting density promotes plant elongation, which increases plant height and produces weaker and unmarkable produce (Taiz et al., 2015).

Optimal plant spacing promotes the effective utilization of solar radiation and nutrients, ensuring healthy growth of plant shoots and roots (Bozorgi et al., 2011). Different cultivars exhibit unique responses to changes in planting density regarding productivity and resource use efficiency (Zhang et al., 2021). In controlled environment agriculture, where the available cultivation area is limited due to the higher cost of production per unit area, understanding the productivity capacity of different cultivars under various planting densities becomes more crucial (Takahashi and Cardoso, 2014). Higher planting density results in a reduction and reduced plant size of maxi-type lettuce and an increase in plant spacing results in larger plants with wider lettuce heads (Cecílio Filho et al., 2007; de Moraes Echer et al., 2001; Mondin et al., 1989; Silva et al., 2000). Reduced plant spacing might increase plant etiolation due to competition between plants for light, reducing the chances of lettuce head formation (Taiz and Zeiger, 2004).

In a controlled environment for lettuce production, the space constraint can be addressed by selecting a suitable cultivar that can produce marketable heads in limited space. Mini-head lettuce allows high-density planting in a limited area, facilitating a short growing cycle and high marketable heads compared to the maxi lettuce. Takahashi and Cardoso (2014) reported that higher planting densities were profitable for producing mini-head lettuce as they require less space for producing marketable produce (cvs. Tudela, Renoir, and Sartre). This cultivar-based cultivation proved beneficial as the production cost per unit area is higher in the controlled environment production facility, emphasizing the need to investigate the effect of increased planting density to evaluate the performance of different lettuce cultivars in a controlled environment hydroponic system. The influence of plant spacing on plant morphology and yield response of different lettuce cultivars are evaluated in this study. The overall objective of this experiment was to 1) investigate the effects of plant spacing on plant growth and development 2) evaluate the romaine lettuce ability to form a head in high density spacing, and 3) identify a romaine lettuce cultivar for optimal growth in a controlled environment.

4.2 Materials and methods

4.2.1 Experimental design and site description

This experiment utilized a randomized complete block design with three factors. Two romaine lettuce cultivars (blocks; cvs. Valley Heart and Breen) were evaluated to determine the effect of spacings (treatment) on plant yield and morphology over three temporal cycles. The treatments included staggered and non-staggered spacing pattern, resulting in a total of eight treatments (A – 6.3 cm, B – 7.6 cm, C – 8.8 cm, D – 10.1 cm) A_1 – non-staggered and A_2 – staggered (Figure 4.1).

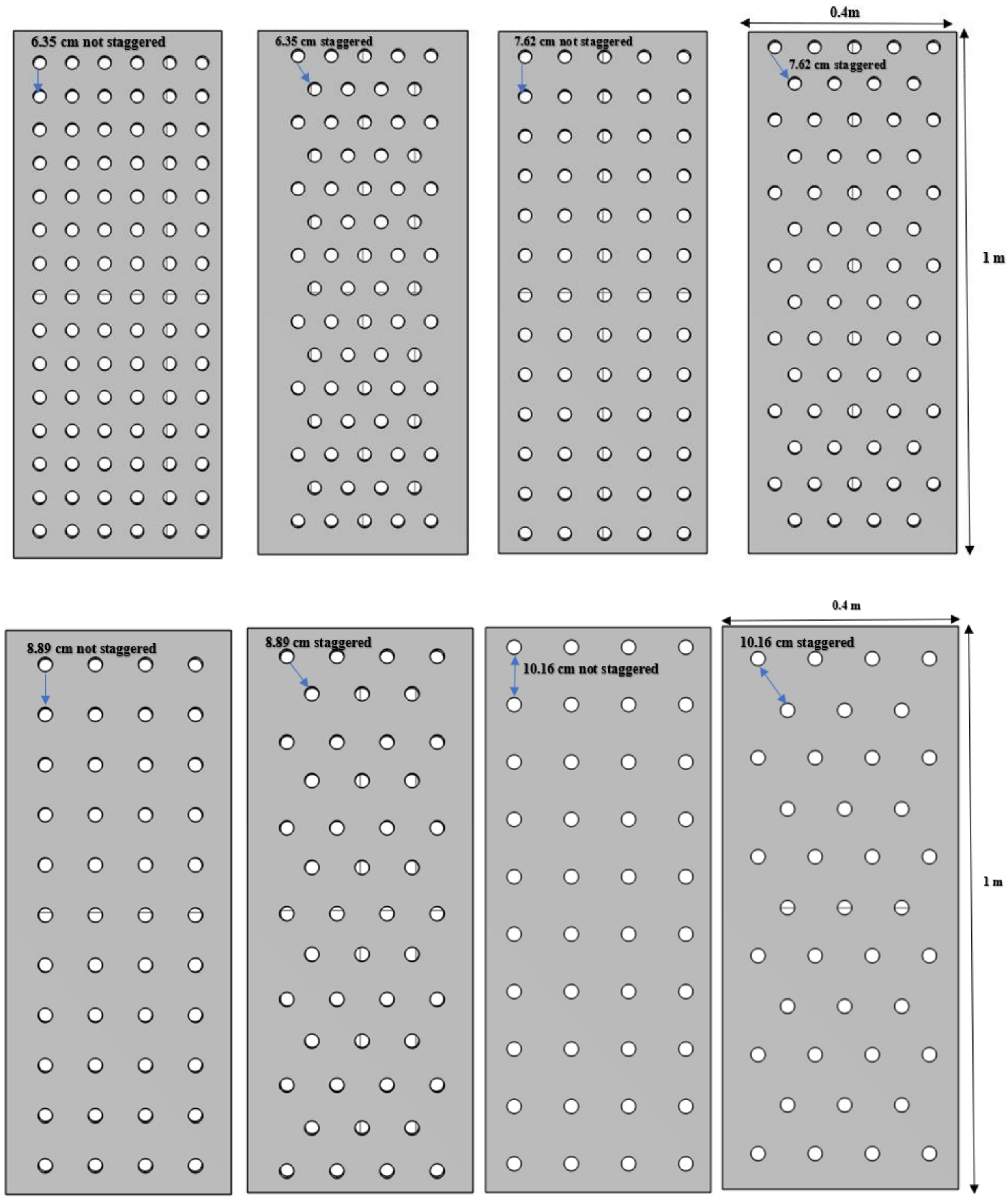

Figure 4.1 Schematic representation of the experimental plant spacings and patterns used in this study (6.3 cm non-staggered, 6.3 cm staggered, 7.6 cm non-staggered, 7.6 cm staggered, 8.8 cm non-staggered, 8.8 cm staggered, 10.1 cm non-staggered, 10.1cm staggered).

The treatments were randomized within each block. This experiment was carried out at McGill University's Macdonald campus research greenhouse (Montreal, Quebec, Canada, N 45° 24' 28.5768", W 73° 56' 19.7916") between February 2022 and December 2022. HPS lights (P.L. Light Systems, Ontario, Canada) were used during the experiment, and their spectral distribution, measured with a spectroradiometer (Apogee PS-300, Minnesota, United States) is depicted in Figure 4.2.

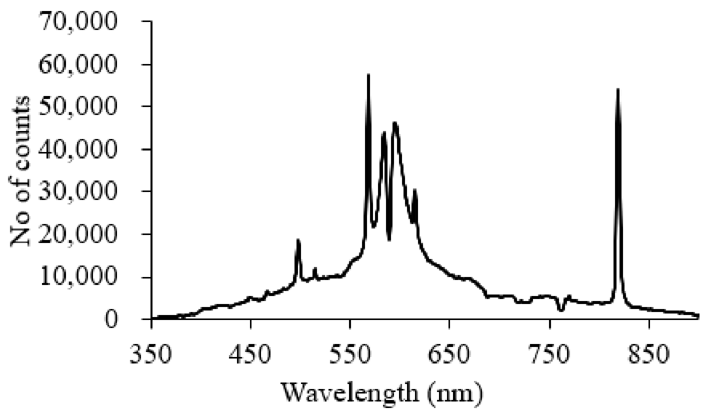

Figure 4.2 High pressure sodium (HPS) spectrum with broad wavelength used in the greenhouse measured using the spectroradiometer.

An aspirator box (Priva B.V., De Lier, Netherlands) was used to constantly monitor the air temperature and humidity levels in the greenhouse. To ensure uniform airflow, horizontal air flow (HAF) fans (Schaefer, North Carolina, US) were utilized to improve the transpiration rate, thereby reducing the tip burn incidence in the romaine lettuce. Monthly average canopy temperature, relative humidity, light intensity, water temperature, and electrical conductivity (EC) of the hydroponic solution which were measured during the experiment are presented in (Table 4.1).

Table 4.1 Controlled environmental conditions for romaine lettuce cultivars. HPS: high-pressure lighting; PPFD: photosynthetic photon flux density.

Abiotic factors	Growth chamber (Days 0–21)	Greenhouse (Days 21–70)
Type of lighting	Fluorescent bulbs	Sunlight and HPS
Light spectrum	400–500 nm	400–700 nm
Photoperiod (Day/Night) (h)	16/8	16/8
PPFD (μmol m^{-2} s^{-1})	100	200
Temperature (Day/Night) (°C)	20/16	20/16
Relative humidity (%)	60±5	55±5
Water temperature (°C)	22	22
Hydroponic solution	Full strength Hoagland's solution 14 days after seeding	Full strength Hoagland's solution from the day 21 after seeding
pH	6.0 ± 0.5	6.0 ± 0.5
Electrical conductivity (μS cm^{-1})	1000 ± 500	1000 ± 500

4.2.2 Planting material

Valley Heart and Breen romaine lettuce (Stokes Seed Ltd. (Thorold, ON, Canada) were double-seeded separately in rockwool cubes (Grodan, Milton, ON, Canada) and placed in a walk-in growth chamber to increase the germination rate (Conviron, BDW80, Manitoba, Canada). After germination, one seedling per rockwool was retained in both varieties by thinning out the germinated seedlings. During the first two weeks, tap water was used for irrigation. After 14 days, a full-strength Hoagland solution (Hoagland and Arnon, 1950) was used to promote faster seedling growth. Three weeks after seeding, the lettuce seedlings were transplanted to ebb-and-flow hydroponic trays (Qualiplast, Quebec, Canada) in the research greenhouse (McGill University's Macdonald campus, Sainte-Anne-de-Bellevue, Canada). For nutrient supply, a plastic bin (Rubbermaid, Atlanta, Georgia, United States) was used to store the nutrient solution, while a submersible water pump (Ecoplus, Austin, Texas, US) was employed to deliver the nutrient solution to the ebb-and-flow system. To reduce salt accumulation and deposition in the nutrient tank, which can potentially increase the EC and total dissolved salts (TDS), the nutrient solution was replaced biweekly. To monitor the nutrient levels in the hydroponic system, water samples were periodically collected and sent for analysis (A&L Canada Laboratories, London, Ontario, Canada).

4.2.3 Environmental monitoring system

Raspberry Pi 4 Model B (Trxcom, Shenzhen, China) was used as an automated agricultural system to monitor environmental parameters in the greenhouse. To measure temperature and relative humidity, a SHT30 sensor (Sincere & Promise, Shenzhen, China) and a I2C communication protocol was used with a temperature range of 0 to 65 °C and a humidity range of 0 to 100 %. To monitor light intensity, the VEML7700 (Adafruit, New York, US) was used with ambient light digital 16-bit resolution and a light range of 0 to 2220 μmol m^{-2} s^{-1}. Root temperatures were monitored with a one wire communication sensor DS18B20 (Maxim, California, US) that ranged from 55 °C to 125 °C. The sensors were connected to the Raspberry Pi system and environmental data was collected every 3 minutes. The pH (0 to 14) and the EC (0 to 3999 μS cm^{-1}) of the hydroponic solution were measured manually by using HI98129 (Hanna, Rhode Island, US).

4.2.4 Harvest

Both the romaine cultivars were harvested 70 days post-seeding, and both the cultivars (Valley Heart and Breen) were subjected to phenotypic characterization by selecting 8 representative samples from each treatment from both cultivars in a randomized manner. Fresh mass, dry mass, plant height, internodal distance, head diameter, and heart diameter were measured. To calculate dry mass, harvested plants were placed in an isotemp oven (Fisher, New Hampshire, US) and dried at 65 °C for 72 h or until a constant dry mass was attained. The plant height was measured with a ruler (Eboot, Massachusetts, US) to measure the distance between the base to the tip of the plant. The internodal distance was assessed to evaluate the risk of bolting under high-density planting conditions. To measure internodal elongation in plants, the distance between the 4th and 5th internodes was measured from the apical meristem as it is the portion where the stem elongation begins. The diameter of the romaine lettuce heads was measured using a vernier caliper (Neotech, Kowloon, Hong Kong), with the scale positioned at the center of the head. The diameter of the heart was measured after removing the head leaves. Starting from the base of the plant and moving upward along the stem, the leaves were counted. The point where the curvature of the leaves changed from curving away from the center of the plant to curving towards the center of the plant was identified as the starting point of the heart-forming leaves, while the leaves which curved away from the center of the plants were identified as the head leaves.

4.2.5 Statistical analysis

Data were analyzed using randomized complete block design with split plot design with SAS 9.4 software (SAS Institute, Cary, NC). Statistical analyses were conducted to determine the effect of block (two cultivars), independent treatment effect (plant spacing and staggered/non-staggered), interaction between treatments, and the treatment-block interaction effect on the plant's responses such as fresh mass, dry mass, plant height, internodal distance, head and heart diameter, head, and heart leaves. Means and standard deviations (SD) of the measured values were determined using analysis of variance (ANOVA). Fisher's Protected LSD test at the 5 % level of significance was used to compare treatment means.

Table 4.2 Effect of plant spacing and pattern on Breen yield. Values are reported as mean ± SE. Sample size n=8. Means in a column followed by the same letter are not significantly different (P>0.05), using Fischer's LSD test.

Treatments	Fresh mass (g)	Dry mass (g)	Plant height (cm)	Internodal distance (cm)	Head diameter (cm)	Heart diameter (cm)
A_1	45.1 ± 3.8^{a}	2.0 ± 0.5^{ab}	31.7 ± 0.6^{a}	1.9 ± 0.1^{a}	0 ± 0.07^{a}	0 ± 0.2^{a}
A_2	50.0 ± 3.8^{a}	2.4 ± 0.5^{b}	30.9 ± 0.6^{ab}	2.0 ± 0.1^{a}	0 ± 0.07^{a}	0 ± 0.2^{a}
B_1	62.4 ± 3.8^{b}	2.7 ± 0.5^{bc}	30.8 ± 0.6^{ab}	1.5 ± 0.1	0 ± 0.07^{a}	0 ± 0.2
B_2	65.7 ± 3.8^{b}	2.5 ± 0.5^{bd}	29.6 ± 0.6^{bc}	1.2 ± 0.1^{b}	0 ± 0.07^{a}	0 ± 0.2^{a}
C_1	83.6 ± 3.8^{cd}	3.6 ± 0.5^{a}	26.7 ± 0.6^{ef}	0.9 ± 0.1^{c}	7.3 ± 0.07	4.8 ± 0.2^{b}
C_2	92.9 ± 3.8^{d}	3.8 ± 0.5^{cd}	28.2 ± 0.6^{ce}	1.0 ± 0.1^{bc}	6.6 ± 0.07^{b}	3.8 ± 0.2^{c}
D_1	86.0 ± 3.8^{cd}	3.5 ± 0.5^{ac}	26.2 ± 0.6^{f}	0.6 ± 0.1^{d}	8.1 ± 0.07	4.5 ± 0.2^{b}
D_2	77.0 ± 3.8^{c}	2.8 ± 0.5^{ab}	26.5 ± 0.6^{ef}	0.7 ± 0.1^{cd}	6.8 ± 0.07^{b}	4.0 ± 0.2^{c}

A_1, B_1, C_1, D_1 – 6.3 cm, 7.6 cm, 8.8 cm, 10.1 cm non-staggered respectively.

A_2, B_2, C_2, D_2 – 6.3 cm, 7.6 cm, 8.8 cm, 10.1 cm staggered respectively.

Table 4.3 Effect of plant spacing and pattern on Valley Heart yield. Values are reported as mean ± SE. Sample size n=8. Means in a column followed by the same letter are not significantly different (P>0.05), using Fischer's LSD test.

Treatments	Fresh mass (g)	Dry mass (g)	Plant height (cm)	Internodal distance (cm)	Head diameter (cm)	Heart diameter (cm)
A_1	45.9 ± 3.8^{a}	2.4 ± 0.5^{a}	46.5 ± 0.6	2.5 ± 0.1^{a}	0 ± 0.07^{a}	0 ± 0.02^{a}
A_2	41.6 ± 3.8^{a}	2.5 ± 0.5^{ab}	44.4 ± 0.6^{a}	2.4 ± 0.1^{bc}	0 ± 0.07^{a}	0 ± 0.02^{a}
B_1	77.7 ± 3.8	3.8 ± 0.5^{ac}	43.5 ± 0.6	2.4 ± 0.1^{a}	0 ± 0.07^{a}	0 ± 0.02^{a}
B_2	64.2 ± 3.8	4.7 ± 0.5^{cd}	41.1 ± 0.6^{b}	1.9 ± 0.1^{c}	0 ± 0.07^{a}	0 ± 0.02^{a}
C_1	95.3 ± 3.8^{b}	4.9 ± 0.5^{cd}	39.1 ± 0.6^{c}	1.9 ± 0.1^{c}	0 ± 0.07^{a}	0 ± 0.02^{a}
C_2	110.8 ± 3.8^{c}	5.0 ± 0.5^{cd}	41.2 ± 0.6^{b}	1.9 ± 0.1^{c}	0 ± 0.07^{a}	0 ± 0.02^{a}
D_1	111.1 ± 3.8^{c}	5.7 ± 0.5^{d}	37.3 ± 0.6^{ac}	1.9 ± 0.1^{c}	0 ± 0.07^{a}	0 ± 0.02^{a}
D_2	93.6 ± 3.8^{b}	3.97 ± 0.5^{bc}	39.3 ± 0.6^{bc}	1.5 ± 0.1^{b}	0 ± 0.07^{a}	0 ± 0.02^{a}

A_1, B_1, C_1, D_1 – 6.3 cm, 7.6 cm, 8.8 cm, 10.1 cm non-staggered respectively.

A_2, B_2, C_2, D_2 – 6.3 cm, 7.6 cm, 8.8 cm, 10.1 cm staggered respectively.

4.3 Results

4.3.1 Fresh mass

This research study aimed to investigate the effects of varying plant spacing and planting patterns on yield and morphological characters of Breen and Valley Heart cultivars. The relationship between plant spacing and bolting, and the correlations between yield and plant spacing, were also studied during this experiment. In this experiment, the yield and plant morphology for both romaine lettuce cultivars were influenced by the plant spacing and planting pattern (Table 4.2 & 4.3). An increase in spacing increased the shoot fresh mass of Breen up to a spacing of 8.8 cm (Figure 4.1). In Breen cultivars, spacing distances of 6.3 cm, 7.6 cm, and 8.8 cm with staggered pattern led to a yield increase of 10.2 %, 5.1 %, and 10.5 %, respectively, compared to non-staggered spacing conditions (Table 4.2). Staggering had a significant effect on fresh mass ($p < 0.05$) on 7.6 cm and 8.8 cm plant spacings in Breen cultivars (Table 4.2). In Breen lettuce increasing the plant spacing from 6.3 cm to 8.8 cm increased the yield up to 69.1 %. Spacing of 10.1 cm reduced the yield in both staggered and non-staggered condition by 7.6 % and 18.7 % when compared to 8.8 cm staggered, which recorded a highest fresh mass yield of 92.9 g. Valley Heart plant spacing had a significant effect ($p < 0.05$) on fresh mass yield (Table 4.3). Staggering the planting pattern for Valley Heart reduced fresh mass yield by 9.8 %, 19 %, and 17 % in spacings 6.3 cm, 7.6 cm, 10.1 cm, respectively, when compared to the subsequent non-staggered spacing pattern (Table 4.3). Staggered spacing of 8.8 cm resulted in an enhancement of the yield for Valley Heart by 15 % compared to the non-staggered spacing condition. Staggered plant spacing of 8.8 cm increased the yield by 15 %. The highest fresh mass of 111.1 g was observed in Valley Heart from plant spacing of 10.1 cm non-staggered (Table 4.3).

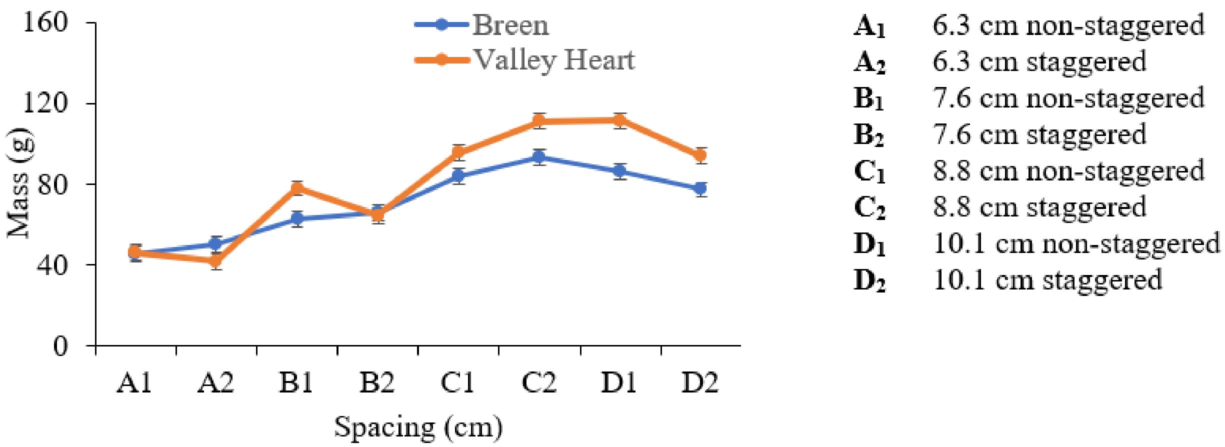

Figure 4.1 Fresh yield of the two different romaine cultivars in different spacing conditions.

4.3.2 Dry mass

The lowest dry mass (2.0 g) was observed for the Breen cultivar under reduced a spacing condition of 6.3 cm non-staggered which increased with increased spacing (Table 4.2). Staggered planting pattern increased the yield in Breen cultivars in spacings of 6.3 cm, and 8.8 cm (Figure 4.2). The highest dry yield (3.8 g) was observed in 8.8 cm non-staggered planting pattern. For the Breen cultivar, a yield reduction of 7.6 % and 22.2 % was observed in 7.6 cm and 10.1 cm staggered spacing conditions respectively compared to the dry yield of non-staggered planting pattern (Table 4.2). Although staggering the planting pattern for Breen influenced the dry yield, it did not have a significant effect ($p > 0.05$) (Table 4.2). The dry mass of the Valley Heart cultivar at the lower spacing condition of 6.3 cm both staggered and non-staggered produced yield less than 3 g (Table 4.3). Staggered the planting pattern increased the dry mass of the Valley Heart cultivar by 4.1 %, 21.1 % and 2 % in 6.3 cm, 7.6 cm, and 8.8 cm plant spacing, respectively, when compared to their non-staggered spacing condition (Figure 4.2). For the Valley Heart cultivar, the 10.1 cm non-staggered planting pattern resulted in the highest dry yield of 5.7 g (Table 4.3), which was significantly higher ($p < 0.05$) than the staggered pattern (Table 4.3). Staggering the planting pattern to 10.1 cm reduced the yield by approximately 37.5 % when compared to its respective non-staggered spacing (Table 4.3).

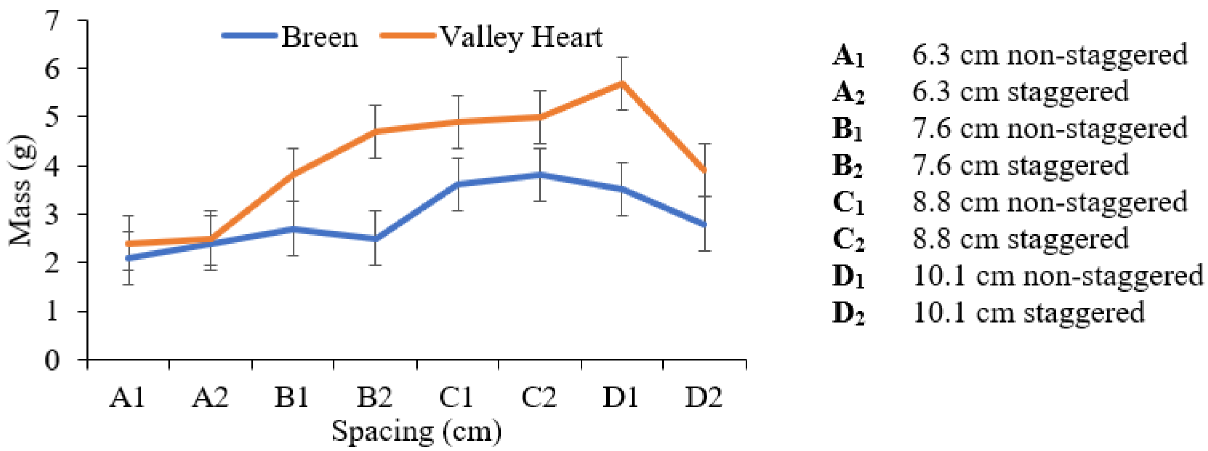

Figure 4.2 Dry yield of the two romaine cultivars in different spacing conditions.

4.3.3 Plant height and internodal distance

Plant spacing had a significant effect ($p < 0.05$) on plant height for both Breen and Valley Heart (Table 4.2 & 4.3). Staggered planting patterns with 6.3 cm and 7.6 cm spacing reduced plant height by 2.5 % and 3.9 % for Breen, and 4.6 % and 5.6 % for Valley Heart, compared to non-staggered spacing (Figure 4.3). However, staggered spacing with 8.8 cm and 10.1 cm increased the plant height by 5.2 % for Valley Heart, and 5.4 % and 1.1 % for Breen, respectively (Figure 4.3). The highest plant height of 31.7 cm for Breen and 46.5 cm for Valley Heart was observed with a non-staggered spacing of 6.3 cm (Table 4.2 & 4.3). The lowest plant heights of 26.2 cm and 37.3 cm were observed for Breen and Valley Heart, respectively, with a higher spacing of 10.1 cm and a non-staggered planting pattern (Table 4.3). Although staggering influenced the plant height in Breen, the staggered planting at a spacing of 6.3 cm, 7.6 cm, 8.8 cm, and 10.1 cm did not demonstrate a significant difference ($p > 0.05$) in plant height compared to the non-staggered planting at the same spacing (Table 4.2). However, the combination of plant spacing and staggering pattern in Valley Heart did show a significant effect ($p < 0.05$) on plant height for 6.3 cm, 7.6 cm, and 10.1 cm spacing, except for 8.8 cm (Table 4.3).

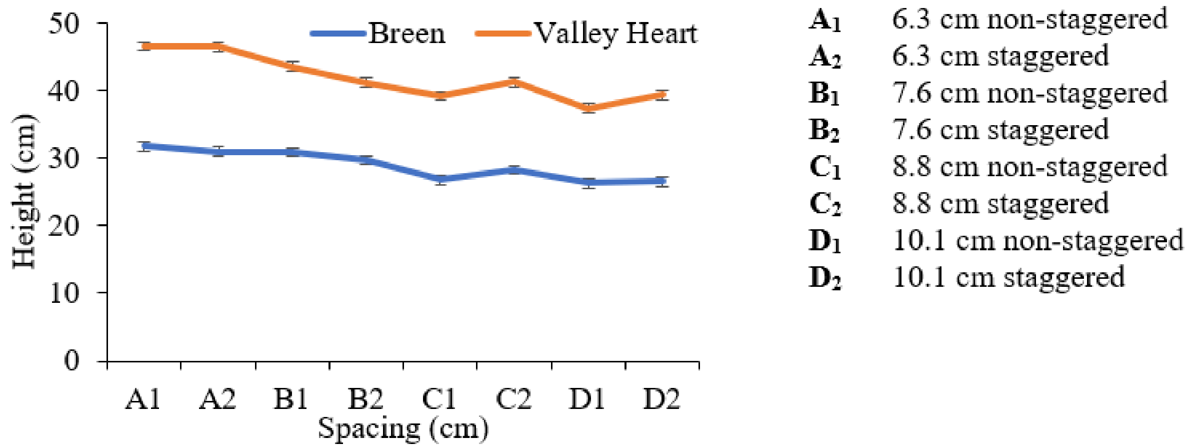

Figure 4.3 Plant height data of the two romaine cultivars under different spacing conditions.

An increase in planting spacing reduced internodal elongation in both romaine lettuce cultivars. For Breen, staggered plant spacing of 6.3 cm, 8.8 cm and 10.1 cm did not have a significant effect ($p > 0.05$) when compared to the same spacing in the non-staggered pattern (Table 4.2). However, staggering had a significant effect ($p < 0.05$) in the 7.6 cm compared to the non-staggered conditions (Table 4.3). The highest internodal elongation of 2 cm was observed with a 6.3 cm staggered spacing condition for the Breen cultivar (Figure 4.4). In Breen cultivar, reducing the number of plants per unit area reduced the internodal elongation with the lowest internodal elongation of 0.6 cm for 10.1 cm non-staggered spacing (Table 4.2). Plant spacing of 8.9 cm and 10.1 cm, both staggered and non-staggered, produced internodal elongation less than and equal to 1 cm (Figure 4.4). Meanwhile, Valley Heart had a significant effect ($p < 0.05$) on staggered planting, with spacing of 6.3 cm and 10.1 cm (Table 4.3). Valley Heart had the highest internodal elongation of 2.5 cm at 6.3 cm non-staggered. For Valley Heart, an internodal distance of 1.9 cm was observed under 7.6 cm staggered, 8.8 cm staggered and non-staggered and 10.1 cm non-staggered (Table 4.3). A lowest internodal distance of 1.5 cm was observed in 10.1 cm staggered spacing pattern for Valley Heart (Figure 4.4). Reducing plant spacing from 6.3 cm non-staggered to 10.1 cm non-staggered reduced the internodal elongation by 92.3 % in Breen and 50 % in Valley Heart lettuce, respectively (Figure 4.4).

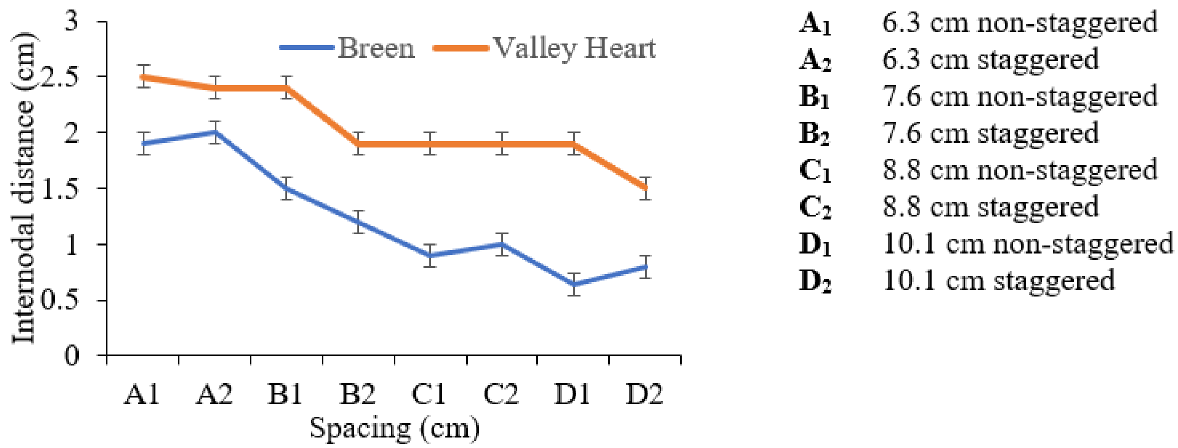

Figure 4.4 Internodal distance of Breen and Valley Heart under different spacing conditions.

4.3.4 Head and heart formation

Head and heart formation in Breen lettuce was observed under 8.8 cm and 10.1 cm spacing with both staggered and non-staggered planting patterns (Figure 4.5). No head and heart formation were observed in Breen for lower spacings, such as 6.3 cm and 7.6 cm (Figure 4.5). Staggering significantly ($p < 0.05$) reduced head diameter by 10 % and 17.4 % and heart diameter by 23.2 % and 11.7 % in 8.8 cm and 10.1 cm, respectively, when compared to the same non-staggered pattern (Table 4.2 & 4.3). A maximum head diameter of 8.1 cm was observed in 10.1 cm non-staggered, and a heart diameter of 4.8 cm was observed in 8.8 cm non-staggered (Table 4.2). The lowest head and heart diameter of 6.6 cm and 3.8 cm were observed under 8.8 cm staggered (Table 4.2). No head and heart development were observed in Valley Heart (Figure 4.5). Even higher spacing conditions and staggering planting pattern did not have a significant effect ($p > 0.05$) on head and heart development for the Valley Heart cultivar (Table 4.3).

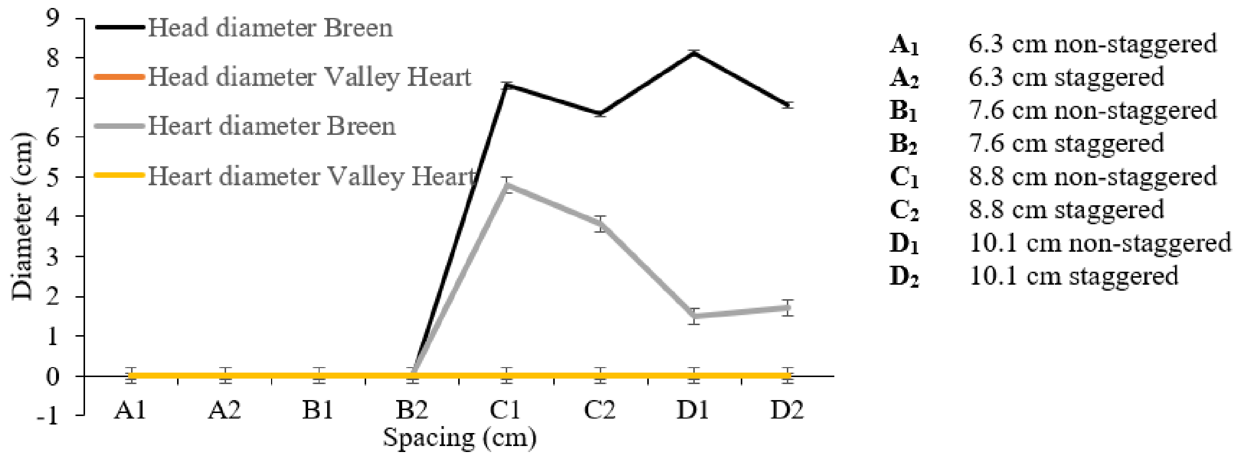

Figure 4.5 Head and heart diameter of Breen and Valley Heart in different spacing conditions.

4.4 Discussion

Planting population density (PPD), known as the number of plants per unit area, influences the vegetative growth and development. Higher PPD can produce interplant competition, therefore, it is important to consider its effects, along with the spatial distribution, as it determines the yield (Tetio-Kagho and Gardner, 1988; Willey and Heath, 1969). Different spacing conditions and planting patterns were followed in this experiment to determine their effects on romaine lettuce yield and morphology. Increasing the plant spacing increased the yield in both cultivars. Fresh and dry mass of both cultivars increased with an increase in spacing. The significant differences in fresh mass in both the cultivars confirmed that reducing planting density of both the cultivars grown in a controlled environment hydroponic system increased the plant fresh mass. However, the yield plateau for both cultivars was not reached because of the experimental design. Despite a lack of significance in dry yield of Breen between different spacing and planting patterns, there was a difference between an average yield of 2.0 g (6.3 cm non-staggered) and 3.5 g (6.3 cm staggered). In both cultivars, 10.1 cm staggered spacing was the only planting pattern that reduced the fresh and dry mass while the other staggered spacings (6.3 cm, 7.6 cm, and 8.8 cm) increased the yield when compared to their other non-staggered spacing.

Plant leaf arrangement and internodal distance are important factors in determining effective light interception for plant photosynthesis (Charles–Edwards, 1982; Monteith, 1969). Increasing the plants per unit area likely increased the competition for light for photosynthesis for both cultivars. For Breen, the lower plant spacings (6.3 cm, 7.6 cm) with both staggered and non-staggered planting pattern resulted in plant etiolation and resulted in lodging. The same trend was observed for the Valley Heart cultivar in which plant etiolation was observed for all spacing tested. This is in agreement with previous findings that an increase in plant density increased plant height (Tetio-Kagho and Gardner, 1988). An increase in plant height promoted the internodal elongation for both cultivars. This study's results align with previous research conducted by Papadopoulos and Ormrod (1991), which demonstrated that lower plant spacing will lead to a significant increase in both plant height and internodal distance, reaffirming the relationship between plant spacing and these growth parameters. With increased internodal elongation, lodging was observed for both cultivars at the lower spacing conditions of 6.3 cm and 7.6 cm with staggered and non-staggered planting pattern. Closer spacing can promote competition for light between plants which results in shading between plants. This induces a plant morphogenic response of increased internodal elongation indicating plant etiolation which is undesirable for plant production(Knight and Mitchell, 1983; Monteith, 1969) . In this experiment, once the internodal distance was less than or equal to 1 cm, the head and heart development was noticed in Breen. Higher plant height and internodal elongation did not promote the head and heart development in both romaine cultivars. Although staggering the planting pattern reduced the internodal elongation in Valley Heart, it did not promote head and heart formation. An increase in plant height and internodal elongation with reduction in plant spacing can be attribute to yield loss (Papadopoulos and Ormrod, 1991).

The lack of a significant difference in head and heart development indicates that different spacing and planting patterns did not contribute to head and heart formation for the Valley Heart cultivar. For the Breen cultivar, reduced plant spacing (6.3 cm, 7.6 cm) with both staggered and non-staggered planting patterns resulted in an elongated lettuce with the lack of a defined head and heart structure. Plant spacing impacts the plant morphology. When the lettuce attains a certain growth stage where the leaves press against each other, further growth tends to be predominantly vertical with increased plant height (Nelder and Moss, 1956). Staggering did not have any effect on head and heart development at lower spacing of Breen (6.3 cm and 7.6 cm) and under all spacing conditions for Valley Heart. Head and heart formation was observed under the spacing ranging from 8.82 cm to 10.1 cm, and both staggered and non-staggered planting patterns were found to promote the development of head and heart in these spacing configurations. Staggered spacing of lettuce resulted in smaller head and heart diameters compared to non-staggered spacing conditions.

4.5 Conclusion

This study demonstrates the importance of lower plant spacing and the effect of different spatial distribution on yield and morphology of different lettuce cultivars in a controlled environment hydroponic system. The combined effect of internodal elongation and plant height on lettuce morphology was determined in this study. Findings from this study will be helpful to select a better performing romaine lettuce variety under lower spacing conditions. A combination of optimum plant spacing, planting pattern and suitable cultivar can produce better yield in available spacing.

Connecting text

Chapters 3, and 4 detailed the research conducted to enhance and optimize the plating density of romaine lettuce cultivars in controlled environment hydroponic system. Chapter 5 provides a summary and discussion of the significant findings.

Chapter 5 General discussion

This research project aimed to optimize and increase the yield efficiency of romaine lettuce by identifying the optimum plant spacing in controlled environment hydroponic system. To cultivate a viable plant population and increase yield output, plant row spacing plays a critical role (Bednarz et al., 2005). As planting density can significantly impact the crop yield, it is crucial in deciding production volume (Bayley, 2020).

This study investigated the effect of different planting densities and planting pattern on the yield and morphological characteristics of romaine lettuce, specifically Valley Heart lettuce, in a controlled environment hydroponic system. The experiment involved various spacing conditions, both staggered and non-staggered, ranging from 5.0 cm to 17.7 cm. Results showed that increasing plant spacing improved the yield of Valley Heart lettuce, with the fresh mass reaching a plateau at a spacing of 20 cm and stabilizing at 25 cm. Beyond 25 cm spacing, increasing the plant spacing did not result in additional yield. Therefore, the identified yield plateau for Valley Heart lettuce corresponds to a planting density of 25 cm. Increasing plant spacing from 5.0 cm to 17.7 cm had a significant positive impact on both fresh mass and dry mass yields. Staggering the planting pattern did not significantly affect the fresh mass yield. Similar trends were observed for dry mass yield. Dry mass yield plateau was observed at 17.7 cm. Planting pattern and density had a significant effect on plant height and internodal elongation. Reducing the planting density from 5.0 cm to 17.7 cm resulted in a reduction in both plant height and internodal elongation. Staggering the plant spacing did not significantly impact plant height or internodal elongation. The formation of lettuce heads and hearts was observed in spacing conditions of 15.2 cm and 17.7 cm, with non-staggered configurations promoting the largest head and heart diameters. Lower spacing conditions did not promote head and heart formation, and staggering the planting pattern did not significantly affect head and heart formation. Increasing plant spacing from 15.2 cm to 17.7 cm resulted in an increase in the number of head and heart leaves. Reduced spacing conditions did not significantly impact head and heart leaf formation, and staggering the plant spacing did not have a significant effect.

Cultivar screening experiment was conducted to examine the effects of plant spacing (6.3 cm, 7.6 cm, 8.8 cm, 10.2 cm) and planting patterns (staggered and non-staggered) on the yield and plant morphology of two romaine lettuce cultivars: Breen, and Valley Heart. This study aimed to determine how different planting configurations would impact the growth and productivity of the lettuce plants. The findings of the study revealed that increasing the distance between the lettuce plants had a positive influence on shoot fresh mass. This means that when the plants were spaced farther apart, they exhibited greater shoot fresh mass, indicating improved growth and development. Increasing the plant spacing led to an increase in shoot fresh mass of Breen up to a spacing of 8.8 cm. Staggered planting patterns resulted in higher yields compared to non-staggered spacing conditions. For Valley Heart, staggering reduced fresh mass yield compared to non-staggered spacing, except for a spacing of 8.8 cm. The lowest dry mass was observed in Breen at a spacing of 6.3 cm non-staggered, which increased with increased spacing. Staggered planting patterns increased the yield in Breen at spacings of 6.3 cm and 8.8 cm. Valley Heart showed increased dry mass with staggered planting at spacings of 6.3 cm, 7.6 cm, and 8.8 cm. Plant spacing had a significant effect on plant height, with staggered spacing reducing plant height for both cultivars at certain spacings. Internodal elongation decreased with reduced plant spacing. The highest internodal elongation was observed in Breen at a spacing of 6.3 cm staggered, while Valley Heart had the highest internodal elongation at a spacing of 6.3 cm non-staggered. Head and heart formation in Breen lettuce were observed at spacings of 8.8 cm and 10.1 cm with both staggered and non-staggered planting patterns. Valley Heart did not show significant head and heart development even with different spacing and planting patterns.

Overall, the research concluded that optimizing plant spacing and adopting specific planting patterns could enhance the yield and growth of romaine lettuce cultivars. These findings contribute to our understanding of plant spacing strategies in lettuce cultivation and can inform farmers and growers in optimizing their planting practices to maximize productivity.

Chapter 6: Conclusion and future studies

6.1 General conclusion

The studies examining the effects of plant spacing and planting patterns on romaine lettuce cultivars helps us to understand the importance of plant spacing on yield and plant morphology. Adjusting the distance between lettuce plants was found to have a significant impact on their growth and productivity. Increasing the spacing between plants resulted in improved shoot fresh mass, indicating better overall growth and development. Moreover, the planting patterns employed also played a crucial role in lettuce yield. Staggering the plant spacing at specific distances demonstrated the highest shoot fresh mass compared to other spacing configurations tested. Plant height and internodal length of the romaine lettuce were interrelated. Head and heart formation was never observed in lower spacing conditions which led to plant bolting and internodal elongation which exhibits the competition existing between the plants in lower spacing condition. Once this competition between the plants is reduced the head and heart formation was observed in romaine lettuce cultivars. Increased planting density also increased the incidence of tip burn in romaine lettuce. Higher planting densities promoted plants with morphological disorder and poor marketable quality. Overall, these findings emphasize the importance of optimizing plant spacing and planting patterns for maximizing the yield and growth of romaine lettuce cultivars. By adjusting the spacing between plants and adopting square planting arrangements, farmers can enhance the growth, yield, and overall quality of their lettuce crops.

Overall, the study emphasizes the importance of selecting appropriate plant spacing to achieve maximum fresh mass production and desired morphological characteristics in Valley Heart lettuce. The findings provide valuable insights for optimizing planting density in controlled environment hydroponic systems, leading to higher and more economically beneficial yields.

6.2 Future suggested studies

The following suggestions are developed based on the results obtained throughout the course of the research. Further knowledge is required on the:

1. The impact of high planting density on plant disease and pests needs further investigation. Understanding how close spacing affects the incidence and severity of diseases and pests will enable the development of effective management strategies to mitigate potential risks.
2. The economic viability of high planting density systems should be assessed. Cost-benefit analyses considering factors such as seed costs, labor requirements, and potential yield gains are necessary to determine the profitability of adopting high-density planting practices.
3. Study the effect of planting density and planting pattern on plant hormones in romaine lettuce cultivars.
4. Evaluate the performance of various romaine lettuce cultivars under different planting densities. By studying multiple cultivars, a broader understanding of their adaptability and suitability for different planting densities can be gained, enabling growers to select the most suitable cultivar for their specific production goals and conditions.
5. Effects of varying environmental conditions, such as temperature, humidity, and light levels, influence the performance of romaine lettuce under high planting density.

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