EFFECTS OF LAMBDA-CARRAGEENAN ON PLANT GROWTH OF BANANA (*Musa acuminata* cv. Berangan)

By

THYE KAH LOK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

November 2023

FBSB 2023 9

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EFFECTS OF LAMBDA-CARRAGEENAN ON PLANT GROWTH OF BANANA (*Musa acuminata* cv. Berangan)

By

THYE KAH LOK

November 2023

Chair : Dhilia Udie Lamasudin, PhD
Faculty : Biotechnology and Biomolecular Sciences

Banana (*Musa acuminata*) is an important food crop in the world because of its role as a staple food and income source for many countries, including Malaysia. Current agrochemical and organic manure practice in commercialized banana production has been a major threat to the environment. Overuse of chemical and manure fertilisers can lead to water, soil, and air pollution, with a consequent negative impact on ecosystems and human health. To address this issue, the development of an alternative environmentally friendly approach is crucial to reduce the usage of chemical and organic fertilisers and promote banana plant growth. Therefore, this study was undertaken to investigate the potential of λ-carrageenan as a plant growth enhancer in *Musa acuminata* cv. Berangan via concentration study (ranging from 0 to 1500 ppm), gene expression analysis using quantitative real-time polymerase chain reaction (RT-qPCR), biochemical assays, nutrient ion analysis, and metabolomics analysis. The application of 750 ppm λ-carrageenan was determined as the optimum concentration as it gave the optimum growth performance on banana plantlets with enhanced plant height, root length, pseudostem diameter, and fresh weight. The RT-qPCR study revealed that gene expressions such as chlorophyllide *a* oxygenase, ribulose-1,5-bisphosphate carboxylase, S-adenosylmethionine synthase, trans-cinnamate 4-monooxygenase, and catalase genes were induced in optimum treatment. However, expression of the class III peroxidase gene, which is involved in hydrogen peroxide accumulation, remained at a low level upon treatment with optimum λ-carrageenan. Similarly, biochemical assays of total chlorophyll content, rubisco activity, total protein content, total phenol content, catalase content, peroxidase content, hydrogen peroxide content, and malondialdehyde content were in parallel with the gene expression analysis. Interestingly, metabolomics profiling analysis also revealed an increase in the abundance of several metabolites involved in carbohydrate metabolism, lipid metabolism, and amino acid biosynthesis in optimum treatment. Moreover, supplementation of optimum λ-carrageenan showed a significant increment of macronutrient uptake (N, P, K, Mg) in banana plantlets. Taken together, the findings suggested that λ-carrageenan at 750 ppm promotes banana plant growth via enhancement of cell metabolism, nutrient uptake, and maintenance of cell homeostasis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN LAMBDA-KARAGENAN TERHADAP PERTUMBUHAN POKOK PISANG (*Musa acuminata* cv. Berangan)

Oleh

THYE KAH LOK

November 2023

Pengerusi : Dhilia Udie Lamasudin, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Pisang (*Musa acuminata*) merupakan tanaman makanan yang penting di dunia kerana peranannya sebagai makanan ruji dan sumber pendapatan kepada banyak negara, termasuk Malaysia. Amalan agrokimia dan baja organik semasa dalam pengeluaran pisang yang dikomersialkan telah menjadi ancaman utama kepada alam sekitar. Penggunaan baja kimia dan baja secara berlebihan boleh mengakibatkan pencemaran air, tanah, dan udara, dengan kesan negatif akibatnya terhadap ekosistem dan kesihatan manusia. Untuk menangani isu ini, pembangunan pendekatan alternatif yang mesra alam adalah penting untuk mengurangkan penggunaan baja kimia dan organik, dan menggalakkan pertumbuhan tanaman pisang. Oleh itu, kajian ini dijalankan untuk menyiasat potensi λ-karagenan sebagai penambah pertumbuhan dalam *Musa acuminata* cv. Berangan melalui kajian kepekatan (antara 0 hingga 1500 ppm), analisis pengekspresan gen menggunakan kaedah tindak balas berantai polimerase kuantitatif masa nyata (RT-qPCR), ujian biokimia, analisis ion nutrien, dan analisis metabolomik. Penggunaan 750 ppm λ-karagenan ditentukan sebagai kepekatan optimum kerana ia memberikan prestasi pertumbuhan yang optimum pada pokok pisang dengan peningkatan ketinggian tumbuhan, panjang akar, diameter batang pseudo, dan berat segar. Kajian RT-qPCR mendedahkan bahawa ekspresi gen seperti klorofilid *a* oksigenase, ribulosa-1,5-bisfosfat karboksilase, S-adenosilmetionina sintetase, trans-sinamat 4-monooksigenase, dan katalase telah diaruh dalam perlakuan optimum. Walau bagaimanapun, ekspresi gen peroksidase kelas III, yang terlibat dalam pengumpulan hidrogen peroksida, kekal pada tahap rendah atas perlakuan λ-karagenan yang optimum. Begitu juga, ujian biokimia untuk jumlah kandungan klorofil, aktiviti rubisco, jumlah kandungan protein, jumlah kandungan fenol, kandungan katalase, kandungan peroksidase, kandungan hidrogen peroksida, dan kandungan malondialdehid adalah selari dengan analisis pengekspresan gen. Menariknya, analisis pemprofilan metabolomik juga mendedahkan peningkatan kelimpahan dalam beberapa metabolit yang terlibat dalam metabolisme karbohidrat, metabolisme lipid, dan biosintesis asid amino dalam perlakuan optimum. Tambahan pula, penambahan λ-karagenan yang optimum menunjukkan peningkatan ketara dalam pengambilan makronutrien (N, P, K, Mg) dalam pokok pisang. Secara keseluruhan, penemuan ini mencadangkan bahawa λ-

karagenan pada 750 ppm menggalakkan pertumbuhan tanaman pisang melalui peningkatan metabolisme sel, pengambilan nutrien, dan penyelenggaraan homeostasis sel.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to everyone who has helped me throughout my academic journey. First and foremost, I extend my heartfelt appreciation to my supervisory committees, Dr. Dhilia Udie Lamasudin, Dr. Zetty Norhana Balia Yusof, and Dr. Lai Kok Song for their invaluable guidance, expertise, and support. Their insightful feedback and encouragement have been significant in the completion of my research and thesis. Special thanks go to Dr. Wee Chien Yeong and Dr. Intan Safinar Ismail for their technical support and assistance. Their knowledge and expertise have been invaluable in overcoming various challenges encountered during my research.

I am grateful to my senior, Wan Muhamad Asrul Nizam Wan Abdullah for his unwavering guidance and assistance. His wealth of experience has greatly aided me in navigating the challenges of our work. I also wish to extend my thanks to my beloved friend, Tang Chu Nie for being a constant source of support and motivation throughout our academic journey, from the very beginning to the end, through all the ups and downs.

Thank you to all members of Floral Biotechnology Laboratory for their collaboration and assistance. Their willingness to share ideas and provide assistance has significantly contributed to the success of my research. I would also like to acknowledge the UPM Graduate Research Fellowship for providing financial support in my academic journey.

I extend my deepest appreciation to my parents, Mr. Thye Chee Seng and Mrs. Lam Wai San as well as my siblings for their endless support, love, and understanding. I would also like to express my sincere thanks to my boyfriend, Tieo Galen for his continuous love and support. His constant encouragement and belief in my abilities have been a source of motivation throughout this challenging journey. I am truly fortunate to have such a caring and supportive family and boyfriend by my side.

To all those mentioned above, and to anyone else who has contributed to my academic journey, thank you!

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Dhilia Udie Lamasudin, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Zetty Norhana Balia Yusof, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Lai Kok Song, PhD
Assistant Professor
Health Sciences Division
Abu Dhabi Women's College
(Member)

_____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 18 April 2024

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CAO	chlorophyllide *a* oxygenase
CAT	catalase
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GDP	Gross domestic product
HSQC	Heteronuclear single quantum coherence
ICP-OES	Inductively coupled plasma optical emission spectroscopy
JRES	J-Resolved spectroscopy
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
PCA	Principle component analysis
PCR	Polymerase chain reaction
PLS-DA	Partial least squares-discriminant analysis
ppm	Parts per million
PRX	peroxidase
PVP	Polyvinylpyrrolidone
qPCR	Real-time polymerase chain reaction
RBCL	ribulose bisphosphate carboxylase, large subunit
ROS	Reactive oxygen species
RT	Reverse transcription
rubisco	Ribulose bisphosphate carboxylase/oxygenase
SAMS	S-adenosylmethionine synthetase
TCA	Trichloroacetic acid
TCM	Trans-cinnamate 4-monooxygenase
TRR	Thioredoxin reductase
TRX	Thioredoxin
TSP	Trimethylsilyl propanoic acid
UBQ	ubiquitin
VIP	Variable importance for the projection

CHAPTER 1

INTRODUCTION

1.1 Background study

The global population hit 8 billion in 2022 and is estimated to reach 9.6 billion by 2050. Consequently, global food production will need to increase by more than 50% to accommodate the global food demand (Searchinger et al., 2019; United Nations, 2013). *Musa acuminata*, commonly known as banana, is the fourth most important food crop in the world after rice, wheat, and maize. Every year, over 100 million tons of bananas are produced and consumed all over the world (Evans et al., 2020). Banana is famous as a staple food for its high nutrient content and affordable price. Besides that, banana is also an important source of income and export revenue for many developing countries, including Malaysia. Bananas are grown by local smallholder farmers and commercial plantations. Local farmers grow and sell banana fruits on a small scale to generate income for living. Meanwhile, commercial plantations create employment opportunities for thousands of people. In 2021, Malaysia exported a total amount of US$ 12.6 million of bananas (OEC, n.d.). Hence, banana is not only a key crop for food security but also a cash crop for local and national economy.

In the past few decades, the demand for bananas has increased exponentially in response to the fast global population growth. The fast-growing population has put pressure on food security and agriculture. To address this issue, utilisation of chemical and organic fertiliser in agricultural land is inevitable to improve crop production. However, excessive use of these fertilisers has created a negative impact on the environment and ecosystem (Kyakuwaire et al., 2019; Chandini et al., 2019). Water runoff carries the excess fertiliser into the lakes, rivers, and other water bodies, causing water pollution. Water consumption from contaminated sources causes harmful effects on human health such as diarrhoea, skin diseases, and cancer (Keena et al., 2022; Lin et al., 2022). Moreover, the polluted water bodies created an unfavourable condition for aquatic life, leading to the death of aquatic life and affecting the entire biodiversity. Aside from water pollution, overuse of chemical and organic fertilisers also contributes to air pollution, where these fertilisers release greenhouse gases, for example, nitrous oxides and carbon dioxide, and resulted in global warming and climate change (Chandini et al., 2019; Zhang, 2011). In addition, organic manure fertilisers produce odour, microorganisms, and other pollutants such as volatile organic compounds, ammonia, hydrogen sulphide and particulate matter, which may pose threats to human health (Keena et al., 2022; Zhang, 2011).

The development of an alternative sustainable agricultural practice is crucial to improve plant growth, meanwhile, to reduce the environmental impact of agrochemicals.

Utilisation of plant growth regulators is one of the most common approaches to enhance plant growth and reduce agrochemical usage. Carrageenan is a natural sulphated polysaccharide obtained from edible red seaweed (Rhodophyta) (Shukla et al., 2016). It is generally categorised into three main classes, namely kappa, iota, and lambda. Over the past century, carrageenan has been widely used in food processing, cosmetic manufacturing, households, medical and pharmaceutical application (Necas and Bartosikova, 2013). At present, carrageenan has been employed to be a potential plant growth enhancer and plant defence elicitor. Carrageenan improved plant growth by stimulating photosynthesis and basal metabolism in *Nicotiana tabacum* (κ-, λ-, ι-carrageenan) (Castro et al., 2012), *Pinus radiata* trees (κ-carrageenan) (Saucedo et al., 2015), and *Eucalyptus* trees (κ-carrageenan) (González et al., 2014). In *Eucalyptus* trees, κ, λ, and ι-carrageenan has also been found to increase the contents of essential oil and polyphenolic compounds, which may enhance protection against pathogens (González et al., 2013). Recently, Pacheco et al. (2021) reported that κ- and ι-carrageenan could stimulate the growth of *Brassica oleracea* seedlings. In addition to enhancing plant growth, κ-, λ-, and ι-carrageenan have also been reported to trigger defence responses against pathogens (Le Mire et al., 2019; Mani and Nagarathnam, 2018; Sangha et al., 2015; Vera et al., 2012; Mercier et al., 2001). By all means, carrageenan has significant potential to act as a dual-functional bio-fertiliser for promoting plant growth and inducing plant defence responses.

Global seaweed production, including wild harvesting and farming, reached 36 million tonnes in 2020 (FAO, 2022). Polysaccharides, such as carrageenan and agar, constitute the most prominent molecules in seaweed cell walls, comprising 40 - 50 percent of their dry weight. Carrageenan can be extracted via several extraction methods, including alkaline treatment, microwave-assisted extraction, enzyme-based extraction, and ultrasound-assisted extraction. Carrageenan yields can vary from 6.5% to 43%, influenced by factors such as seaweed species and extraction conditions (Manuhara et al., 2016; Hilliou et al., 2012). For instance, alkaline extraction from *Chondrus crispus* produced a 58% of λ-carrageenan yield (Pereira, 2013). The natural origin, widespread availability, and biodegradability make it environmentally friendly compared to synthetic chemical fertilisers. In this study, λ-carrageenan was selected for its water solubility and high degree of sulphation. The water solubility property enables λ-carrageenan to dissolve in water and be readily absorbed by plants. Besides, λ-carrageenan exhibits higher eliciting activity in plants due to its high sulphation level (Cunha and Grenha, 2016; Mercier et al., 2001). Furthermore, purified λ-carrageenan was preferred over crude seaweed extract. Crude seaweed extract contains numerous biochemical compounds in varying concentrations, which may impose diverse effects on plant growth. This complexity makes it challenging to identify the specific compound responsible for promoting plant growth or specific mechanisms when utilising crude seaweed extract. In addition, the concentrations of compounds in crude seaweed may vary between batches, leading to inconsistent effects on plant growth. Therefore, applying purified λ-carrageenan allows for better manipulation on the concentration, ensuring more consistent effects on plant growth. This approach facilitates a more precise understanding of the plant growth-promoting mechanism of λ-carrageenan and enables the development of bio-stimulants with targeted and effective concentrations.

Although the incorporation of carrageenan was shown to improve growth in several plants, limited knowledge is known on applying λ-carrageenan to enhance plant growth in food crops, where the banana plant is used in this study. In addition, the knowledge of the underlying mechanism of λ-carrageenan in plant growth promotion remains limited. Thus, this study was undertaken to investigate the effects of λ-carrageenan in enhancing banana plant growth, as well as to elucidate the possible mechanism by which λ-carrageenan promotes the growth of banana plants. Taken together, this study provides a deeper understanding of the plant growth-promoting response induced by λ-carrageenan, which could be beneficial for developing an environmentally friendly fertiliser to improve plant growth and reduce the dependence on chemical fertiliser.

1.2 Objectives

The objectives of the present study were:

1. To evaluate the effects of λ-carrageenan at different concentrations on the growth performance of banana plants;

2. To assess the effects of λ-carrageenan on banana plant growth and defence response *via* gene expression analysis, biochemical assays, and nutrient ion analysis;

3. To investigate the probable growth-promoting mechanism of λ-carrageenan in banana plants.

CHAPTER 2

LITERATURE REVIEW

2.1 Banana (*Musa acuminata* spp.)

2.1.1 General description

Banana (*Musa* spp.) is the largest herbaceous flowering plant, which is grown in tropical and subtropical regions (Sidhu and Zafar, 2018). It is classified in the genus *Musa* from the family of Musaceae and the order of Zingiberales. At present, over 1,000 varieties of bananas are cultivated in the world (Li et al., 2013). Edible banana cultivars are derived from two wild species of *Musa acuminata* (AA) and *Musa balbisiana* (BB) (Stover and Simmonds, 1987). The hybridization events between species or subspecies led to a range of banana varieties being cultivated. Most varieties are generally triploid (AAA, AAB, ABB), but diploid (AA, AB, BB) and tetraploid hybrids (AAAA, AAAB, AABB, ABBB) are rare. Banana is one of the oldest cultivated plants. It is believed to be originated in Southeast Asia, and it is then introduced to other countries for cultivation (Li et al., 2013). Today, banana is cultivated in more than 150 countries (Voora et al., 2023).

2.1.2 Importance of banana

Banana is generally divided into two categories, namely dessert banana and cooking banana (also known as plantain). Banana fruits can be eaten raw or cooked, as well as it can be processed to make chips, flour, purees, wine, vinegar, and more (Aurore et al., 2009). Banana flowers can also be eaten as vegetables (Marikkar et al., 2016). Besides, all parts of banana plants can be applied for medicinal uses. For example, the fruit is used as a remedy for constipation, and the flower is used to treat ulcers and bronchitis (Kumar et al., 2012). Aside from food purposes, other parts of the banana plant can be utilised for non-food purposes such as food wrapping, roofing shelter, rope making, textile manufacturing, and many other applications (Marikkar et al., 2016; Mohiuddin et al., 2014; Kumar et al., 2012).

Banana is popular to be consumed all over the world because of their flavourful taste, nutrient content, and low price. Over 100 billion bananas are consumed worldwide annually (Eco Business Fund, 2022). Banana is rich in carbohydrate, which provides energy to the human body (Kumar et al., 2012). Moreover, banana is also a good source of vitamins and minerals, which is essential for human health (Aurore et al., 2009). Furthermore, banana plays an important role in global food security where it provides

major staple calorie and substantial nutrition for more than 500 million people in developing countries (Voora et al, 2023; Aurore et al., 2009).

In terms of economic value, banana is the fourth most important food crop in the world, where it ranked after rice, wheat, and maize (Alemu, 2017). The global banana production was recorded at 125 million metrics tons in 2021 and is forecasted to reach 133 million tonnes by 2029 (Shahbandeh, 2023; FAO, 2020). In addition, banana is an important income source for many developing countries such as Malaysia, Indonesia, and the Philippines. Banana farming provides livelihoods for many rural households. In Africa, over 70 million people generate income from banana farming (Kole, 2022). Additionally, the commercial banana industry provides numerous job opportunities in developing countries, especially for rural women (FAO, 2017). Besides that, banana is also an important export revenue source for many developing countries, which help improve the nation's economy and GDP growth (Alemu, 2017). Altogether, banana is an important cash crop as it contributes to income generation, poverty alleviation, unemployment reduction, and economic development.

2.1.3 Productivity limitation

Currently, the banana industry has been constrained by several challenges such as climate change, loss of farmland, and poor agricultural practices, pests and diseases, as well as use of fertilisers and pesticides. According to the regional climate-yield model, climate change declined banana production in some countries such as Brazil, Indonesia, Malaysia, and the Philippines from 1961 to 2016 (Varma and Bebber, 2019). Tan (2022) also reported a decline in banana production in Malaysia between 2017 and 2019, attributed to unfavourable environmental conditions and reduced agricultural land. Global production is predicted to be decreased by 2050 due to climate change. Moreover, the higher temperature in some regions increases plant water demand while the decline in rainfall reduces water supply, hence inadequate water conditions will eventually affect banana production (Ahmed Khan et al., 2019; Tinzaara et al., 2018). Besides, the availability of suitable land use is one of the limiting factors in the development of the banana industry (Nuarsa et al., 2018). Large-scale urban expansion, to accommodate the increasing populations, has led to a huge loss of farmland all over the world (Bren d'Amour et al., 2016). Impaired land conditions such as soil infertility and poor moisture retention also affect banana production negatively (Tinzaara et al., 2018). Additionally, banana production is threatened by pests and diseases such as banana weevils, root parasitic nematodes, Fusarium wilt, black Sigatoka, banana bunch top disease, and Moko disease (Abagale et al, 2019; Tinzaara et al., 2018; Viljoen et al., 2017). Many pests and diseases posed a severe banana yield loss of up to 100%, causing food insecurity and economic loss (Okonya et al., 2019). Furthermore, underuse of fertilisers and pesticides led to reduced productivity, undermined soil health, increased pest population, and induced pest resistance (Ritchie et al., 2022; Zhang et al., 2015). On the contrast, overuse of fertilisers and pesticides had threatened food safety, human health and the environment.

2.1.4 Environmental impact of banana production

In the past few decades, the application of chemical and organic fertilisers had overcome some limitations of banana production. For example, nitrogen, phosphorus, and potassium (N:P:K) fertiliser, urea, cypermethrin, carbendazim and manure (Kyakuwaire et al., 2022, Solomon Wisdom et al., 2012; Bhuiyan et al., 2009). High inputs of chemical and organic fertilisers have been applied to banana plantations to achieve high banana yield (Bhuiyan et al., 2009; Chen, 2006). However, the overuse of these fertilisers has caused negative effects on the environment, ecosystem, and human health (Kyakuwaire et al., 2022; Chandini et al., 2019; Chen, 2006; Calderon and Rola, 2003). Excess fertilisers are leached into groundwater, rivers, lakes, and other water sources, contaminating the water supply and polluting the environment. Organic manure, which contains countless microorganisms, including bacteria, fungi, viruses, and parasites, could run off into water sources and cause transmitted diseases in animals and human (Keena et al., 2022). Water eutrophication is one of the major effects caused by excessive agrochemicals and manure. Algal blooms caused by water eutrophication produce toxins and deplete dissolved oxygen in the water bodies, resulting in the killing of aquatic life, contamination of the domestic water supply, and degradation of coastal recreational activities (Keena et al., 2022; Gobler, 2020; Le Moal et al., 2019; Savci, 2012). Loss of aquatic life due to water pollution caused great damage to the aquatic ecosystem and fisheries economics. Poisoning of animals and human health risks such as gastrointestinal illnesses, skin diseases, reproductive disorders, and cancer could be imposed by domestic consumption of polluted water (Lin et al., 2022; Khan et al., 2013). Apart from water pollution, air pollution is also one of the major deleterious effects caused by the intensive use of agrochemicals and organic manure. The application of fertilisers can result in the release of dust, particulate matter, microorganisms, greenhouse gases, and other pollutants, which contribute to poor air quality and climate change (Munsif et al., 2021; Savci, 2012; Zhang, 2011). Consequently, poor air quality increases the risk of respiratory problems, heart disease, stroke, lung cancer, and leukaemia. In addition, imbalanced soil chemistry, loss of organic matter, and soil compaction caused by overuse of agrochemicals can lead to soil degradation and erosion, which eventually results in food insecurity (Savci, 2012; Liu et al., 2010).

2.2 Plant growth

2.2.1 Mechanism of plant growth

Plant growth is a complex and dynamic process that plays an important role in the growth, survival, and reproduction of plants. It is driven by the activity of meristematic cells, which produce new cells that differentiate into various tissues and organs (Kitagawa and Jackson, 2019). This process involves both primary growth, which is characterized by the elongation of shoots and roots, and secondary growth, which results in the stem thickening. Under favourable conditions, plants can undergo continuous growth by increasing their size and structure throughout their life (Hunt 2012). In addition, plants grow in response to various environmental stimuli, such as light, water,

nutrients, and temperature. Plants can adapt to changing environments rapidly due to their plasticity characteristic (Kitagawa and Jackson, 2019; Dubois et al., 2018).

As plants are sessile organisms, they have developed sophisticated mechanisms to utilise various metabolic processes, which support plant growth and development (Dubois et al., 2018). These metabolic processes include photosynthesis, nutrient uptake and utilisation, maintenance of cell homeostasis, and biosynthesis of cellular components, such as carbohydrates, lipids, and proteins. Plant growth is fuelled by the process of photosynthesis, where energy harvested from sunlight is converted into chemical energy in the form of sugars (Gao et al., 2018; Evans, 2013). These sugars are then used as an energy source and as building blocks for the biosynthesis of other cellular components (Slewinski and Braun, 2010). Biosynthesis of cellular components such as proteins, lipids, and phenolics can help plants in regulating cellular processes and maintaining cell homeostasis (Thirumurugan et al., 2018; Goff, 2011; Wang, 2004). Nutrient uptake from the soil is also essential for plant growth. These nutrients are taken up by the roots, transported to the shoot and leaves, and utilised for basic cellular processes (Arif et al., 2016; Maathuis and Diatloff, 2013). Furthermore, cell homeostasis is crucial for proper growth and development in plants, as well as for the survival of the plant under various stress conditions (Mhamdi and Van Breusegem, 2018). Plants have specialized mechanisms to maintain the balance of ions and water, nutrients, and stress response within the cell to prevent cellular damage and support cellular processes (Chapman et al., 2019; Haydon et al., 2015; Bassil et al., 2012).

2.2.2 Plant growth enhancer

Plant growth enhancers are substances, either natural or synthetic, that are used to stimulate plant growth. These substances include fertilisers, plant hormones, microorganisms, and natural extracts. Fertilisers are the most common method being utilised over the world. They provide essential nutrients such as nitrogen and potassium to support plant growth and crop yield (Solomon Wisdom et al., 2012). In addition, plant hormones, also known as plant growth regulators, such as auxin and gibberellin modify various physiological processes in plants, which ultimately improve plant growth and development (Chandini et al., 2019; Ogunyale et al., 2014). Plant growth-promoting microorganisms such as rhizobia and mycorrhizae enhance plant growth by facilitating nutrient acquisition, regulating phytohormones, and improving stress tolerance (Abhilash et al., 2016). In recent years, natural extracts from plants, seaweed, and compost have been studied for their potential to enhance plant growth. As reported previously, the extracts of *Moringa oleifera* leaf improved plant growth and alleviated abiotic stresses in plants (Arif et al., 2023; Soares et al., 2021). Seaweed extracts such as carrageenan and laminarin had been demonstrated to be a plant growth stimulator and plant defence elicitor (Shukla et al., 2019; Khan et al., 2009). Arancon et al. (2006) also revealed that humic substances extracted from compost increased the growth of marigold, pepper, and strawberry plants.

Plant growth enhancer derived from natural sources, such as seaweed extracts or microbial inoculants, can replace chemical fertilisers to increase agricultural productivity by providing essential nutrients and promoting plant growth in a more sustainable and environmentally friendly manner (Arif et al., 2023; Shukla et al., 2019; Arancon et al., 2006, Mercier et al, 2001). The natural origin and biodegradability of these enhancers make them environmentally safer alternatives. Chemical fertilisers often contribute to soil degradation, water pollution, and greenhouse gas emissions. Plant growth enhancers, on the other hand, have minimal environmental impact and can help mitigate the adverse effects associated with conventional farming practices by enhancing soil fertility, microbial activity, and nutrient cycling. For instance, humic acid induces positive effects on plant growth and soil health. It stimulates physiological processes in plants such as nutrient uptake, chlorophyll synthesis, root development, and hormone activity, while also improves soil properties and aids in remediating contaminated soil (Ampong et al., 2022; Li et al., 2019). Utilising natural plant growth enhancers can reduce the reliance on synthetic inputs, enhance plant growth, and minimise adverse environmental effects.

2.3 Carrageenan

2.3.1 General description

Carrageenan is a naturally occurring high molecular weight polysaccharide, which is extracted from edible red seaweed (Rhodophyta) such as *Hypnea musciformis*, *Eucheuma denticulatum*, *Kappaphycus alvarezii* and *Chondrus crispus* (Necas and Bartosikova, 2013). It is commonly found in the Atlantic Ocean near Britain, Europe, and North America (Necas and Bartosikova, 2013). The name carrageenan originated from the Carrageen Moss in England and Carraigin in Ireland, with a meaning of 'little rock' in Irish (Shukla et al., 2016). Over decades, carrageenan has been widely used as hydrocolloids in various industries such as food technology, pharmaceutical, cosmetics, and biotechnology (Abdul Khalil et al., 2017).

2.3.2 Type and structure

Carrageenan is a linear chain of sulphated polysaccharide, with a backbone structure of repeating D-galactose and 3,6-anhydrogalactose residues with alternating α-1,3 and β-1,4-glycosidic linkage (Stadnik and Freitas, 2014). Its ester-sulphate content is within 15% to 40% of its total weight, where its average relative molecular mass is much greater than 100 kDa. It is also water-soluble and possesses a strong negative charge over normal pH. Carrageenan can be classified into various forms, which are iota (ι), kappa (κ), lambda (λ), mu (μ), theta (θ), and nu (ν), and their structures are displayed in Figure 1. The number and position of ester sulphate group, as well as the occurrence of 3,6-anhydrogalactose residues, make the main differences between carrageenan types (Li et al., 2014). Necas and Bartosikova (2013) reported that a lower level of ester sulphate group leads to lower solubility temperature and lower gel strength. Despite, the exception of β-carrageenan is due to lacking sulphate content (Cunha and Grenha, 2016). Among

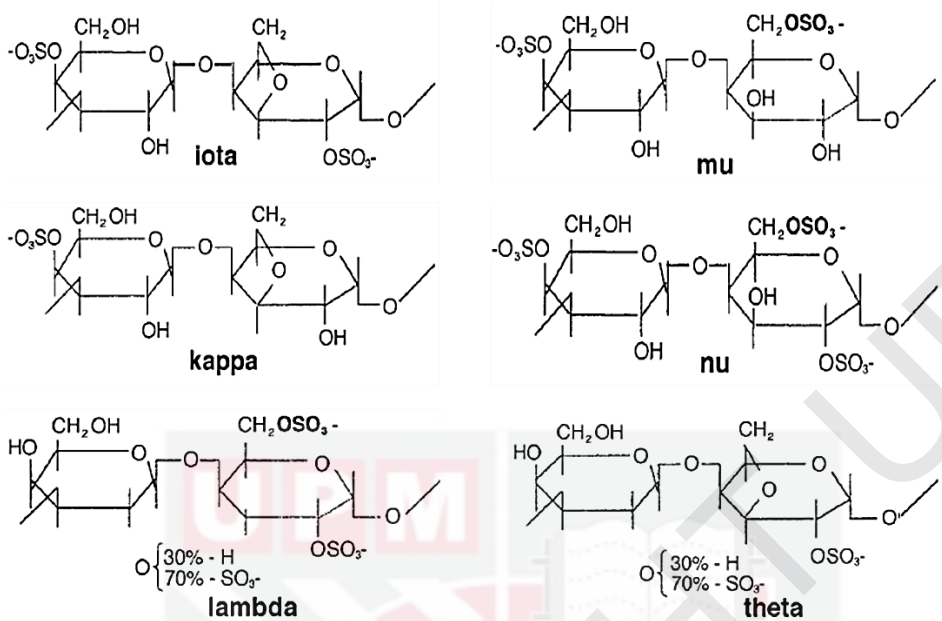


Figure 1: Classification of carrageenan and its chemical structures. (Adapted from Imeson, 2000)

all carrageenan types reported, the most commercially important types are iota (ι), kappa (κ), and lambda (λ). The comparison of these main types of carrageenan is tabulated in Table 1. The κ-carrageenan is made of D-galactose sulphated at the C4 position linked with anhydrogalactose, where it contains one sulphate group per repeating unit. The ι-carrageenan is formed of D-galactose sulphated at the C4 position linked with anhydrogalactose sulphated at the C2 position, thus it has two sulphate groups per repeating unit. Whilst λ-carrageenan is made of D-galactose sulphated at the C2 position linked with D-galactose sulphated at C2 and C6 positions, thereby it has three sulphate groups per repeating unit (Shukla et al., 2016; González et al., 2013). Among these three major types, λ-carrageenan contains the highest level of ester sulphate group which is 32% to 39%, compared to ι-carrageenan with 28% to 30% and κ-carrageenan with 25% to 30% (Cunha and Grenha, 2016). Cunha and Grenha (2016) also reported that ι-carrageenan contains 25% to 30% of 3,6-anhydrogalactose residues, and κ-carrageenan has 28% to 35% of 3,6-anhydrogalactose residues; however, λ-carrageenan has no 3,6-anhydrogalactose content. Different carrageenan types exhibit different abilities in gelling. The λ-carrageenan does not form a gel, where its structure of having more sulphate groups does not allow it to form a double helix. Meanwhile, ι- and κ-carrageenan can form gel, by allowing the formation of double helices and binding the chain molecules in a three-dimensional network (Cunha and Grenha, 2016; Li et al., 2014). In addition, carrageenan is water soluble but insoluble in most inorganic solvents. The solubility of carrageenan is dependent on its hydrophilicity, where the higher the hydrophilic molecule the more soluble it is. The hydrophilicity is provided by the sulphate and hydroxyl groups, while the 3,6-anhydrogalactose shows hydrophobic. The λ-carrageenan exhibits more hydrophilic, which is easily soluble in water, due to its three sulphate groups and lack of 3,6-anhydrogalactose. Although the presence of hydrophobic 3,6-anhydrogalactose residues in ι-carrageenan, it possesses a moderate solubility since it has two hydrophilic sulphate groups. However, κ-carrageenan, with the structure of 3,6-anhydrogalactose and only one sulphate group per repeating unit, is less soluble (Cunha and Grenha, 2016).

Among the types of carrageenan, λ-carrageenan has the highest level of sulphation due to the presence of three sulphate groups per repeating unit. The high degree of sulphation enables it to exhibit strong eliciting activity in plants and other organisms, effectively stimulating various physiological processes (Cunha and Grenha, 2016; Mercier et al., 2001). As previously reported by Mercier et al. (2001), a higher level of ester sulphate group in carrageenan efficiently improves signalling and defence mechanism in plants. The high sulphation level also prevents λ-carrageenan from forming a double helix, thus it does not exhibit a gel-like form (Li et al., 2014). In addition, λ-carrageenan is the most soluble type of carrageenan compared to κ- and ι-carrageenan. The presence of three sulphate groups and absence of 3,6-anhydrogalactose makes λ-carrageenan more hydrophilic, allowing it to dissolve easily in water and be easily absorbed. This properties makes it a promising candidate as a bio-stimulant for promoting plant growth and eliciting defence responses.

Table 1: Comparison of the main types of carrageenan.

Type of carrageenan	Position of sulphate group	Number of sulphate group per repeating unit	Level of ester sulphate group	Level of 3,6-anhydrogalactose residues	Gel formation	Solubility in water
κ-	D-galactose sulphated at the C4 position linked with anhydrogalactose	1	25% - 30%	28% - 35%	Form gel	Less soluble
ι-	D-galactose sulphated at the C4 position linked with anhydrogalactose sulphated at the C2 position	2	28% - 30%	25% - 30%	Form gel	Moderate soluble
λ-	D-galactose sulphated at the C2 position linked with D-galactose sulphated at C2 and C6 positions	3	32% - 39%	No	Does not form gel	More soluble

2.3.3 Application of carrageenan

Carrageenan has been commercially utilised in many fields, such as the food industry, households, cosmetics, and pharmaceutical applications. The abilities of gelling, thickening, suspending, stabilizing, and emulsifying have brought carrageenan to be greatly utilised in the food industry. It has been employed to act as a stabiliser in ice cream, emulsify soy oil in cheese, and improve the quality of processed meat (Yasin et al., 2016; Rojas-Nery et al., 2015; Bahram-Parvar et al., 2013). Kanmani and Rhim (2014) also suggested that carrageenan-based film can be used as active food packaging due to its antimicrobial properties. Aside from the food industry, it has also been applied in toiletries and cosmetics including toothpaste and shampoo, as well as air freshener gels, shoe polish, firefighting foam, and probiotic capsules (Muhardina et al., 2018; Li et al., 2014). In the past decade, the application of carrageenan in pharmaceuticals is increased due to its potential pharmaceutical properties including anticancer, antiviral, antitumor, antifungal, anticoagulant, anti-inflammatory, antihyperlipidemic, antioxidant, and immunomodulatory activities (Jazzara et al., 2016; Prasedya et al, 2016; Soares et al., 2016; Ahmadi et al., 2015; Luo et al., 2015; Necas and Bartosikova, 2013). Recently, carrageenan has been reported to be a potential elicitor for plant growth and defence response.

2.3.4 Carrageenan on plant growth

Previous reports revealed that carrageenan could be used as a plant growth enhancer in some plant species, including *Cicer arietinum* L. (Bi et al., 2011), *Zea mays* (Bi et al., 2011), *N. tabacum* (Castro et al., 2012; Moenne et al., 2010), *Eucalyptus globulus* (González et al., 2013), *Catharanthus roseus* L. (Naeem et al., 2015), *P. radiata* (Saucedo et al., 2015), *Verbena bonariensis* L. (Salachna et al., 2017), *Arachis hypogaea* (Abad et al., 2018), and *B. oleracea* (Pacheco et al., 2021; Antonio et al., 2019). The κ-, ι-, and λ-carrageenan tends to promote plant growth by enhancing photosynthesis, basal metabolism, cell division (González et al., 2013; Castro et al., 2012; Moenne et al., 2010). Bi et al. (2011) claimed that the application of κ-carrageenan stimulated plant growth in *C. arietinum* L. and *Z. mays* by increasing plant height, stem diameter, and number of leaves for both, and the number of pods and branches for *C. arietinum* L. As reported by González et al. (2014), application of κ-carrageenan induced the synthesis of NADPH, ascorbate, glutathione, and TRR/TRX activity in *Eucalyptus globulus*. This led to the stimulation of photosynthesis and basal metabolism, ultimately promoting plant growth. Besides, Saucedo et al. (2015) also reported that κ-carrageenan improved growth in *P. radiata* trees by increasing carbon, nitrogen, and sulphur assimilation. According to Naeem et al. (2015), physiological activities such as photosynthetic parameters, activities of nitrate reductase, carbonic anhydrase, and tryptophan decarboxylase were improved upon the application of κ-carrageenan. Salachna et al. (2017) also claimed that ι-carrageenan enhanced plant growth parameters such as plant height, number of inflorescences, and number of leaves in *Verbena bonariensis*. In addition, κ-carrageenan was found to shorten the period of germination and flowering as well as improve plant height and pod yield in *A. hypogaea* (Abad et al., 2018). Moreover, Antonio et al. (2019) suggested that carrageenan treatment had significantly enhanced plant growth and yield in *B. oleracea*. Pacheco et al. (2021) also concluded that κ- and

ι-carrageenan could promote the growth of aerial part in *B. oleracea* seedlings. The effects of carrageenan on plant growth are summarized in Table 2.

The application of carrageenan enhances plant growth by modulating important physiological processes in plants. Based on the findings mentioned previously, κ-carrageenan has been reported to induce plant growth by enhancing photosynthesis, basal metabolism, cell division, assimilation of carbon, nitrogen and sulphur, as well as production of secondary metabolites. However, only limited study proposed that ι- and λ-carrageenan promote plant growth through photosynthesis, basal metabolism and cell division. In short, further detailed investigations are needed to unveil the underlying role of ι- and λ-carrageenan in enhancing plant growth.

Table 2: The effect of carrageenan on plant growth.

Plant species	Type of carrageenan	Effects	Reference
Chickpea (*Cicer arietinum* L.)	κ-	Promoted overall plant growth such as plant height, stem diameter, number of pods, branches, and leaves, and days to flowering	Bi et al., 2011
Maize (*Zea mays*)	κ-	Elevated plant height, stem diameter, and number of leaves	Bi et al., 2011
Tobacco (*Nicotiana tabacum*)	κ-, ι-, λ-	Increased plant height, foliar biomass, and number of leaves	Moenne et al., 2010
Tobacco (*Nicotiana tabacum*)	κ-, ι-, λ-	Induced photosynthesis, basal metabolism, and cell division, increased plant parameters such as height and biomass	Castro et al., 2012
Eucalyptus globulus	κ-, ι-, λ-	Enhanced plant growth by improving plant height and trunk diameter, and increased contents of cellulose, essential oils, and polyphenolic compounds	González et al., 2013
Eucalyptus globulus	κ-	Stimulated photosynthesis, basal metabolism, and plant growth by increasing NADPH, ascorbate, and glutathione syntheses, as well as TRR/TRX activity	González et al., 2014
Catharanthus roseus L.	κ-	Enhanced growth traits (number of leaves per plant, average leaf area, fresh and dry weight), physiological activities (photosynthetic parameters, activities of nitrate reductase, carbonic anhydrase, and tryptophan decarboxylase), leaves and herbage yield, and content of alkaloids	Naeem et al., 2015
Pinus radiata	κ-	Increased plant height and induced carbon, nitrogen, and sulphur assimilation	Saucedo et al., 2015

Table 3: Continued

Verbena bonariensis L.	ι-	Improved plant height, number of inflorescences, and number of leaves	Salachna et al., 2017
Peanut (*Arachis hypogaea* L.)	κ-	Improved plant height, reduced period of germination and flowering, and increased pod yield	Abad et al., 2018
Kale (*Brassica oleracea* var. acephala)	Not specified	Enhanced plant growth such as plant height, leaves number and width, root length, plant weight, and leaves weight, as well as increased plant yield in passive hydroponics systems	Antonio et al., 2019
Kale (*Brassica oleracea*)	κ-, ι-	Increased aerial part length and weight	Pacheco et al., 2021

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant material

Musa acuminata cv. Berangan (AAA group) was used in the study. Two-month-old plantlets were bought from the Global Green Tissue Culture Nursery (Klang, Malaysia). The plantlets were grown in polyethylene bags filled with peatmoss soil at 25 ± 2 °C under a long-day condition (16 hours light/ 8 hours dark). The plantlets were irrigated with distilled water three times a week and acclimatised for two weeks before λ-carrageenan treatment.

3.2 Concentration study of λ-carrageenan on banana plants growth

Pure λ-carrageenan powder was purchased from Sigma Aldrich, Germany. In this study, plantlets of *Musa acuminata* cv. Berangan was treated via soil-drench method with λ-carrageenan at a regime of 0, 250, 500, 750, 1000, and 1500 ppm. The plantlets were treated with 50 mL of λ-carrageenan at concentrations of 250, 500, 750, 1000, and 1500 ppm every seven days over a two-week period (Antonio et al., 2019; Salachna et al., 2017; Chakhatrakan, 2003). Plantlets treated with 50 mL distilled water (0 ppm) were used as negative controls. During the experiment, no other fertiliser was added to the plantlets. The experiment was performed in triplicates with three biological replicates (n=3) for each treatment. The morphological appearance of the plantlets and growth parameters such as plant height (cm), root length (cm), pseudostem diameter (cm), and fresh weight (g) was recorded a day before treatment (as initial measurement) and a week after treatment (as final measurement). Leaf samples were collected randomly and stored at -80 °C until further use.

3.3 Quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) analysis

3.3.1 Primer designing

Six target genes involved for respective biochemical assays were selected, namely chlorophyllide *a* oxygenase (*CAO*), ribulose-1,5-bisphosphate carboxylase (*RBCL*), S-adenosylmethionine synthetase (*SAMS*), trans-cinnamate 4-monooxygenase (*TCM*), Class III peroxidase (*PRX*), and catalase (*CAT*) genes. All primers were designed using

Primer-BLAST from the National Centre for Biotechnology Information (NCBI). The primer sequences used for RT-qPCR analysis were listed in Appendix 1.0.

3.3.2 RNA extraction and cDNA synthesis

Total RNA extraction was conducted using RNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. Approximately 100 mg of leaf samples were ground into a fine powder using a chilled pestle and mortar in the presence of liquid nitrogen. Lysis buffer (450 µL) was added to the tissue powder and the mixture was vortexed vigorously. The lysate was transferred to a shredding column and centrifuged at 10,000 × *g* for two minutes at room temperature (24 °C). The supernatant was added to 0.5 volume of 70% (v/v) ethanol and was mixed by pipetting. The mixture was transferred to a spin column and centrifuged at 8,000 × *g* for 15 seconds at room temperature. Washing buffers were added to the spin column and centrifuged to remove biomolecules and salts. The flow-through was discarded. The spin column was centrifuged at 10,000 × *g* for one minute at room temperature to dry the membrane. The RNA was eluted with 50 µL of RNase-free water. The concentration and purity of extracted RNA were determined using Implen Nanophotometer® (Implen GmbH, Germany). The accepted range of 260/280 ratio for RNA purity is 1.8 to 2.0, while the accepted range of 260/230 ratio is 2.0 to 2.2.

Reverse transcription was performed via QuantiNova Reverse Transcription Kit (Qiagen, Germany) according to the manufacturer's instructions. In brief, one µg of extracted total RNA was used for reverse transcription. The extracted RNA was added to the genomic DNA removal mix, make up to a total volume of 15 µL, and incubated for two minutes at 45 °C. The reaction was immediately placed on ice. The reverse transcription master mix was added to the reaction and incubated in Bio-Rad T100TM Thermal Cycler (Bio-Rad Laboratories, USA) as followed: 25 °C for three minutes, 45 °C for 10 minutes, and 85 °C for five minutes. The resulting cDNA for each sample was kept at -20 °C for further analysis.

3.3.3 Optimisation of selected genes amplification

Primer specificity was validated by PCR and gel electrophoresis. Optimisation of gene amplification was performed using QuantiNova SYBR Green PCR Kit (Qiagen, Germany) in Bio-Rad CFX 96™ thermal cycler (Bio-Rad Laboratories, USA). A series dilution of the cDNA (1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000 dilutions) was used to construct a standard cDNA concentration curve. The serial dilution was conducted by mixing one part of the previous dilution with nine parts of distilled water, making up the next dilution. For instance, to make the 1:10 dilution, one part of the cDNA main stock was diluted with nine parts of distilled water; the 1:10 dilution was used to make the 1:100 dilution. The RT-qPCR reaction consisted of five µL 2x QuantiNova SYBR Green PCR master mix, 0.7 µL 100 nM forward primer, 0.7 µL 100 nM reverse primer, one µL

cDNA dilution, and RNase-free water, makeup to a total volume of 10 µL. Melt curve analysis was set from 65 °C to 95 °C, with a 0.5 °C increment for every five seconds to determine the primer specificity. The slope and y-intercept of the standard curve were obtained for each primer pair. The accepted value for the coefficient of determination (R^2) is ranging from 0.985 to 1.000, while the PCR efficiency (E) between 90% and 110% is acceptable. The standard curve and melt curve for each primer pair were shown in Appendix 2.0.

3.3.4 Gene expression analysis of selected genes

The cDNA synthesised in Section 3.3.2 was diluted to 100 ng/µL and used for RT-qPCR analysis. Gene expression analysis was carried out using QuantiNova SYBR Green PCR Kit (Qiagen, Germany) in Bio-Rad CFX 96™ thermal cycler (Bio-Rad Laboratories, USA). A total volume of 10 µL RT-qPCR reaction contained five µL of 2x QuantiNova SYBR Green PCR Master Mix, 0.7 µL of 100 nM forward primer, 0.7 µL of 100 nM reverse primer, one µL of diluted cDNA, and RNase-free water. No template control (NTC) was set up to identify contamination and primer-dimer formation. The cycling conditions for RT-qPCR were set as followed: 95 °C for 2 minutes, followed by 40 cycles of 95 °C for five seconds and 60 °C for 10 seconds. Expression of *CAO, RBCL, SAMS, TCM, PRX*, and *CAT* genes was studied. Two housekeeping genes, namely glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and ubiquitin (*UBQ*), were used as reference genes to normalise the gene expression level of target genes (Wan Abdullah et al., 2022). The experiment was performed in triplicates with three biological replicates (n=3) for each treatment.

3.3.5 Data analysis

The data was analysed in Bio-Rad CFX Maestro 2.2 software (Bio-Rad Laboratories, USA). The relative expression level of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

3.4 Biochemical assays

To investigate the effects of λ-carrageenan on plant growth, samples from control (0 ppm), 250 ppm, optimum concentration (750 ppm) and high concentration (1500 ppm) were selected based on the results obtained from Section 3.2. Optimum concentration was chosen to identify the growth-promoting effects of λ-carrageenan on plant growth. The 250 ppm was selected to serve as a comparison to optimum treatment to determine whether they undergo physiological processes differently. Meanwhile, high concentration was chosen to understand the negative effects of λ-carrageenan at high concentration on plant growth. The experiment was performed in triplicates with three biological replicates (n=3) for each treatment.

3.4.1 Determination of chlorophyll content

The contents of chlorophyll a, chlorophyll b, and total chlorophyll in leaves of banana plantlets treated with different λ-carrageenan concentrations were determined according to Fortunato et al. (2012). Briefly, 200 mg of leaf tissues were ground with a pestle and mortar in the presence of 1 mg/mL calcium carbonate. The ground powder was then homogenised with 2 mL of 80% (v/v) acetone in the dark at room temperature. The homogenate was filtered with Whatman® paper and the residues were washed several times with 80% acetone. The extract was topped up to 25 mL with 80% acetone. The absorbance of 647 and 663 nm were read using a UV/Vis spectrophotometer (Jenway, UK). The contents of chlorophyll a (c_a), chlorophyll b (c_b), and total chlorophyll (c_{a+b}) were calculated as followed and expressed in mg per gram of fresh weight:

$$c_a = 12.25\ (A_{663}) - 2.79\ (A_{647})$$

$$c_b = 21.50\ (A_{647}) - 5.10\ (A_{663})$$

$$c_{a+b} = 7.15\ (A_{663}) + 18.71\ (A_{647})$$

3.4.2 Determination of rubisco activity

Rubisco activity in banana plantlets treated with λ-carrageenan was performed according to Khan et al. (2015). One gram leaf samples were homogenised with ice-cold extraction buffer [0.25 M Tris-hydrocloride acid (Tris-HCl), pH 7.8; 0.05 M magnesium chloride ($MgCl_2$); 0.0025 M ethylenediaminetetraacetic acid (EDTA); 37.5 mg dithiothreitol (DTT)]. After centrifugation at 10,000 × *g* for 10 minutes at 4 °C, the resulting supernatant was added to three mL of reaction mixture [100 mM Tris-HCl, pH 8.0; 40 mM sodium bicarbonate; 10 mM $MgCl_2$; 0.2 mM nicotinamide adenine dinucleotide; 4 mM adenosine triphosphate (ATP); 0.2 mM EDTA; 5 mM DTT; 1 U glyceraldehyde-3-phosphodehydrogenase; 1 U 3-phosphoglyceratekinase; 0.2 mM ribulose-1,5-bisphosphate]. Absorbance was measured spectrophotometrically at a wavelength of 340 nm.

3.4.3 Quantification of total protein content

Total protein content was quantified as reported by Bradford (1976). Bradford reagent (BioRad, USA) was prepared by diluting the concentrated solution with sterile distilled water at a ratio of 1:4. Roughly 150 mg of leaves were homogenised in 1.5 mL of 10 mM potassium phosphate buffer (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone (PVP), and were centrifuged at 12,000 × *g* for 30 minutes at 4 °C. The resulting supernatant (20 µL) was then mixed with one mL of diluted Bradford reagent. After 5-minute incubation, absorbance was read at a wavelength of 595 nm. The standard curve was plotted using bovine serum albumin as a standard. The total protein content was determined by comparing to the standard curve and was expressed in unit µg per gram of fresh weight.

3.4.4 Determination of total phenolic content

Quantification of total amounts of phenolic compounds in banana plantlets was performed using Folin-Ciocalteu method described by Dallagnol et al. (2011), with minor modification. Leaf tissues (100 mg) were ground using a pestle and mortar and homogenised with 1.5 mL of 80% (v/v) methanol. After overnight incubation in the dark with shaking, the homogenate was centrifuged at 12,000 × *g* for five minutes at room temperature. The supernatant (150 µL) was added to one volume of 0.25 N Folin-Ciocalteu reagent and kept at room temperature for five minutes. One hundred fifty milliliters of 1 M sodium carbonate was added and incubated at room temperature for 10 minutes. Distilled water was mixed with the reaction mixture, followed by one-hour incubation at room temperature. Then, the absorbance of 725 nm was recorded. Gallic acid was used to prepare the standard curve. Results were expressed as mg of gallic acid equivalents (GAE) per gram of fresh weight.

3.4.5 Hydrogen peroxide quantification

Accumulation of hydrogen peroxide (H_2O_2) levels in banana plantlets was determined according to Velikova et al. (2000). Briefly, leaf samples (200 mg) were homogenised with 2 mL of 0.1% (w/v) trichloroacetic acid (TCA). After centrifugation at 12,000 × *g* at 4 °C for 15 minutes, the supernatant (500 µL) was added to 500 µL of 10 mM potassium phosphate buffer (pH 7.0) and one mL of 1 M potassium iodide. The absorbance of 390 nm was measured using a UV/Vis spectrophotometer (Jenway, UK). The value was compared with the standard curve plotted using known concentrations of H_2O_2. The hydrogen peroxide content was calculated and expressed in µM per gram of fresh weight.

3.4.6 Malondialdehyde assay

Lipid peroxidation levels in the leaf samples were measured by malondialdehyde (MDA) assay according to Kok et. al. (2021). Two grams of leaves were ground in a pestle and mortar with 10 mL of 10% (w/v) TCA, followed by centrifugation at 12,000 × *g* for 15 minutes at room temperature. An equal volume of solution containing 10% (w/v) TCA and 0.6% (w/v) thiobarbituric acid was added to the supernatant. The mixture was incubated in a water bath for 20 minutes at 100 °C and was left for cooling. Then, the mixture was centrifuged at 12,000 × *g* for 10 minutes at room temperature. Absorbance was recorded at 532, 600, and 450 nm. The MDA content was calculated using the formula as followed and expressed as nmol per gram fresh weight:

$$\text{MDA content} = 6.45\ (A_{532} - A_{600}) - 0.56\ (A_{450})$$

3.4.7 Detection of catalase activity

Catalase assay was conducted on the leaf samples according to Velikova et al. (2000). Leaf samples (150 mg) were ground using a chilled pestle and mortar and homogenised with 1.5 mL of 10 mM potassium phosphate buffer (pH 7.0) containing 4% (w/v) PVP, followed with centrifugation at 12,000 × *g* for 30 minutes at 4 °C. The crude enzyme (100 µL) was mixed with three mL of catalase reaction consisting of 10 mM potassium phosphate buffer (pH 7.0) and 3% (w/v) H_2O_2. The absorbance of 240 nm was taken over three minutes. An extinction coefficient of 40 mM^{-1} cm^{-1} was used to calculate the catalase activity. The value was expressed as unit per mg protein, where one unit is the amount of enzymes that catalyses the decomposition of 1 µmol H_2O_2 per minute.

3.4.8 Peroxidase assay

Peroxidase activity in banana plantlets treated with λ-carrageenan was carried out according to Fortunato et al. (2012). Approximately 250 mg of leaf tissues were homogenised in 2 mL of 100 mM potassium phosphate buffer (pH 6.8) containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 300 mg polyvinylpolypyrrolidone. After centrifugation at 12,000 × *g* for 15 minutes at 4 °C, the supernatant (100 µL) was added to three mL of assay mixture [100 mM potassium phosphate buffer (pH 6.8), 100 mM pyrogallol, and 100 mM H_2O_2]. Absorbance was monitored using a UV/Vis spectrophotometer (Jenway, UK) at 420 nm over three minutes. The peroxidase activity was calculated using the extinction coefficient (2.47 mM^{-1} cm^{-1}) and expressed as unit per mg protein.

3.5 Nutrient ion analysis

To assess the effects of λ-carrageenan on nutrient uptake in banana plantlets, samples from control (0 ppm), optimum concentration (750 ppm) and high concentration (1500 ppm) were selected based on the results obtained from Section 3,3 and 3.4. Optimum concentration was selected to examine the effects of λ-carrageenan on nutrient uptake, while high concentration was chosen to assess the potential negative effects on nutrient uptake. The experiment was performed in triplicates with three biological replicates (n=3) for each treatment.

3.5.1 Determination of nitrogen by Kjeldahl method

Leaf samples were harvested and dried at 65 °C for 72 hours. Roughly one g of leaf tissues was ground into a fine powder using a pestle and mortar. The powdered sample was added to 20 mL of concentrated sulphuric acid and 2 g of catalyst mixture (selenium and sodium sulphate), mixed gently, and left for 30 minutes. The sample was digested

for three hours until the solution becomes clear. After cooling, the sample was diluted with 50 mL of distilled water and transferred to the distillation unit. The sample was then mixed with 50 mL of 40% sodium hydroxide. During the distillation process, the distillate was collected in 75 mL of 2% boric acid-indicator solution and titrated with 0.1 N hydrochloric acid until the colour of the solution changed. The experiment was performed in triplicates with three biological replicates (n=3) for each treatment. Nitrogen content was calculated as followed:

Nitrogen content (g/100 g) =

$$\frac{(\text{Volume}_{\text{sample}} - \text{Volume}_{\text{blank}}) \times \text{Normality of acid} \times \text{Atomic weight of nitrogen} \times 100}{\text{Weight of sample} \times \text{Dry residue}}$$

3.5.2 Inductively coupled plasma optical emission spectroscopy (ICP-OES)

Leaf samples were pre-rinsed with deionised water and were dried at 65 °C for 72 hours. The dried samples were ground into a fine powder using a pestle and mortar. The powder was then added to hydrochloric acid: nitric acid solution at a ratio of 4:1, followed by the digestion process in a microwave oven (CEM Mathews, NC, USA). The digested samples were left to cool and diluted with deionised water. The sample was then analysed in Avio® 500 ICP optical emission spectrometer (PerkinElmer, USA) for P, Ca, K, Mg, Fe, Mn, and Zn. The experiment was performed in triplicates with three biological replicates (n=3) for each treatment. The operating parameters for ICP-OES were listed in Appendix 3.0.

3.6 Metabolomics analysis via nuclear magnetic resonance (NMR) spectroscopy

3.6.1 Sample preparation and metabolite extraction

The extraction procedure was conducted according to Hidalgo et al. (2016). Approximately 25 mg of leaf samples were ground using a chilled pestle and mortar in liquid nitrogen until turned into powder. The tissue powder was dissolved in 800 µL deuterated methanol and 200 µL of potassium dihydrogen phosphate buffer in deuterium oxide (pH 6.0), containing 0.05% trimethylsilyl propanoic acid (TSP). The TSP was used as an internal reference. The mixture was vortexed for one minute and sonicated for 15 minutes at room temperature. The homogenate was then centrifuged at 11,000 × g for 10 minutes at room temperature. The supernatant (600 µL) was transferred to a 5-mm NMR tube (Norell, USA) for NMR analysis.

3.6.2 NMR analysis

The ^{1}H NMR spectra were acquired using a 500 MHz Varian INOVA NMR spectrometer (Varian Inc., USA) at room temperature. Deuterium oxide was used as an internal lock. The total acquisition time for ^{1}H NMR spectrum was 3.53 minutes, using 64 scans with a width of 20 ppm. The 2D NMR spectra such as J-resolved (JRES) and heteronuclear single quantum coherence (HSQC) were obtained using Bruker 700 MHz spectrometer (Bruker, MA, USA) at room temperature. The spectra were acquired using 24 scans, 1 k data points, 256 increments at a spectral width of 16 ppm in dimensions and a relaxation delay of 2 seconds.

3.6.3 Data processing

The NMR spectra were manually phased, baseline corrected, and calibrated to the internal standard (TSP) at 0.00 ppm using Chenomx NMR Suite 5.1 Professional software (Chenomx Inc., AL, Canada). The chemical shift region of 0.0 to 10.0 ppm was reduced to an integrated bucket of 0.04 ppm width. The spectral regions for residual water (4.7-4.9 ppm) were removed. The remaining spectral regions were normalised to the total sum of the spectral intensity. The binned data of ^{1}H NMR spectra were exported into excel for further analysis.

3.6.4 Multivariate data analysis

The binned data of ^{1}H NMR spectra were analysed using SIMCA 14.1 software (Umetrics, Umea, Sweden). The datasets were mean-centred and Pareto-scaled before multivariate analysis via Principle Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) models. Data were visualised with the score plot of two principle components (PC1 and PC2), in which each point represented an individual spectrum of a sample. The metabolites associated with the group separation were indicated by the corresponding loading plots, in which each point represented a single NMR spectral bin. The validation and fitting of the generated model were assessed by using the computation of R^2Y and Q^2 and permutation test.

3.7 Statistical analysis

Data collected were analysed using GraphPad Prism 8.0.1 software (GraphPad Software, San Diego). All data and graphs presented were expressed as means ± standard error (SE) of triplicates (n=3). Significant differences were determined using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests, where *p*-value less than 0.05 was considered to be statistically significant.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Concentration study of λ-carrageenan on banana plantlets

In this study, optimisation of λ-carrageenan concentration was conducted on banana plantlets treated with a range of 0, 250, 500, 750, 1000, and 1500 ppm λ-carrageenan via soil drench method. These treatments aimed to evaluate the effect of λ-carrageenan at different concentrations on the growth performance of banana plantlets morphologically and determine the optimum concentration for banana plant growth. The growth performance of banana plantlets treated with different concentrations of λ-carrageenan was recorded in Figure 2. In general, all replicates of control plantlets and plantlets treated with 250, 500, 750, and 1000 ppm λ-carrageenan were green and healthy (Figure 2a-e) whereas most of the banana plantlets treated with 1500 ppm (8 out of 9 plantlets) showed stunted growth and yellowing of leaves (Figure 2f).

Differences between initial and final measurements of plant height, root length, pseudostem diameter, and fresh weight of banana plantlets were recorded in Figure 3. As compared to control (0 ppm), an increment in growth performance was observed in all λ-carrageenan treatments, except a concentration of 1500 ppm. A significant increment in plant height was recorded in 750 ppm treatment, where it had grown 0.611 ± 0.07 cm taller upon treatment (Figure 3a). Low λ-carrageenan concentrations notably increased root length and fresh weight. The root length of banana plantlets had increased by 1.78 ± 0.22 cm, 1.96 ± 0.34 cm, 2.52 ± 0.15 cm, and 2.13 ± 0.30 cm in 250, 500, 750, and 1000 ppm λ-carrageenan, respectively (Figure 3b). Meanwhile, the increment of fresh weight was recorded at 1.63 ± 0.31 g, 1.79 ± 0.32 g, 2.03 ± 0.49 g, and 1.71 ± 0.33 g in 250, 500. 750, and 1000 ppm λ-carrageenan, respectively (Figure 3c). On the other hand, the treatment with 1500 ppm had a lower increment in fresh weight (0.32 ± 0.19 g) and pseudostem diameter (0.19 ± 0.05 cm) than the control. No remarkable effect was detected on pseudostem diameter in all λ-carrageenan treatments as compared to the control (Figure 3d).

In this study, a gradual improvement in growth parameters was observed with the increasing concentration of λ-carrageenan up to 750 ppm. However, the values declined at 1000 and 1500 ppm of λ-carrageenan (Figure 3). Among all the λ-carrageenan concentrations, the banana plantlets treated with 750 ppm showed the greatest increment in plant height, root length, fresh weight, and pseudostem diameter. This result aligned with previous studies whereby the application of carrageenan resulted in enhanced plant growth such as height, root length, and weight (Antonio et al., 2019; Abad et al., 2018; Salachna et al., 2017; Naeem et al., 2015; Saucedo et al., 2015; González et al., 2013;

Figure 2: Effect of λ-carrageenan at different concentrations on banana plant morphology. Representative images of (a) control plant (0 ppm); (b) 250 ppm; (c) 500 ppm; (d) 750 ppm; (e) 1000 ppm; (f) 1500 ppm. Arrow indicates a yellowing leaf. Scale bar, 5 cm.

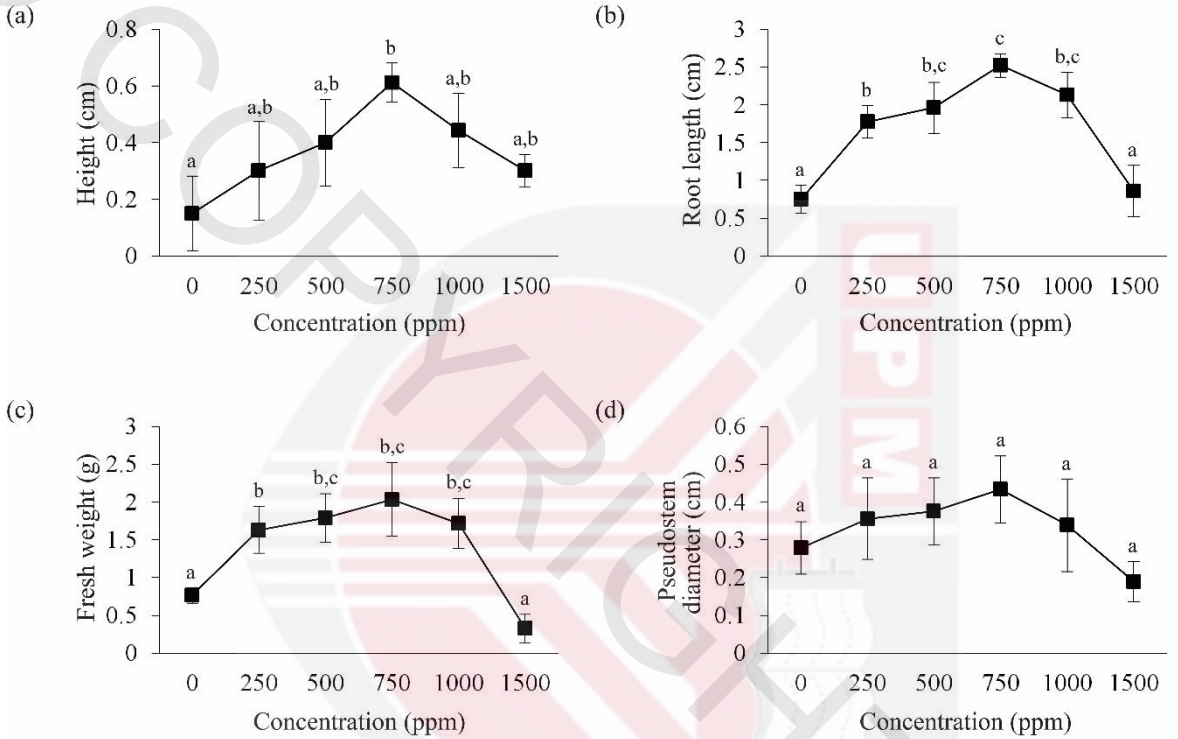


Figure 3: Effect of λ-carrageenan at different concentrations on banana plant growth parameters. (a) Plant height; (b) root length; (c) fresh weight; (d) pseudostem diameter. Results are presented as mean ± SE of three replicates (n=3). Letters (a,b,c) denote significant difference between the control and treatment groups ($p < 0.05$).

Castro et al., 2012; Bi et al., 2011; Moenne et al., 2010). Enhancement in plant growth performance may be attributed to the increased photosynthesis and basal metabolism triggered by carrageenan (Saucedo et al., 2015; Naeem et al., 2015; González et al., 2014; Castro et al., 2012). Increased photosynthesis and basal metabolism could lead to increased energy generation and biomass accumulation in plants, further enhancing cellular metabolism and plant growth, which positively reflects on plant growth parameters.

Foliar spraying at a concentration of 1 mg/mL (equivalent to 1000 ppm) carrageenan improved plant height in *N. tabacum* (κ-, ι-, λ-carrageenan), *Eucalyptus* tree (κ-carrageenan), and *Pinus* tree (κ-carrageenan), as well as plant weight in *N. tabacum* (κ-, ι-, λ-carrageenan) and trunk diameter in *Eucalyptus* tree (κ-carrageenan) (Saucedo et al., 2015; González et al., 2013; Castro et al., 2012). Whilst Naeem et al. (2015) reported that *C. roseus* L. exhibits optimum growth when foliar sprayed with 80 mg/L (equivalent to 80 ppm) κ-carrageenan. Moreover, Bi et al. (2011) revealed that the application of 100 μg/mL (equivalent to 100 ppm) liquid κ-carrageenan around sowing seeds in soil has a better effect in improving plant growth compared to foliar spraying and applying solid formulation in soil. It is plausible that different plant species and application methods may require different doses of carrageenan treatment to achieve optimum growth. Therefore, in this study, a concentration of 750 ppm λ-carrageenan was identified as the optimum concentration for promoting banana plant growth.

4.2 Gene expression analysis of banana plantlets response to λ-carrageenan treatment

Supplementation helps regulate plant growth and development. Due to their sessile nature, plants have evolved complex mechanisms to regulate plant adaptation and optimise growth and productivity (Dubois et al., 2018). These mechanisms included photosynthesis, protein biosynthesis, phenolic compound production, cellular homeostasis maintenance, and other physiological processes. Numerous studies have shown that application of carrageenan exhibited a positive effect on the biochemical changes in plants to enhance plant growth (Naeem et al., 2015; Saucedo et al., 2015; González et al., 2014; González et al., 2013). However, very little research has explored the role of carrageenan in promoting plant growth from the perspective of gene expression. Thus, understanding gene expression patterns in plants is crucial to gain a deeper insight into the effect by which λ-carrageenan promotes plant growth. By combining the insights obtained from both gene expression studies and biochemical assays, this study could provide a more comprehensive and holistic understanding of the growth-promoting mechanism induced by λ-carrageenan.

In this study, expression patterns of six target genes associated with growth promotion and maintenance of cell homeostasis in banana plantlets upon λ-carrageenan treatment were evaluated via RT-qPCR analysis (Figure 4). The target genes were selected based on the corresponding biochemical assays listed in Section 3.4. These target genes

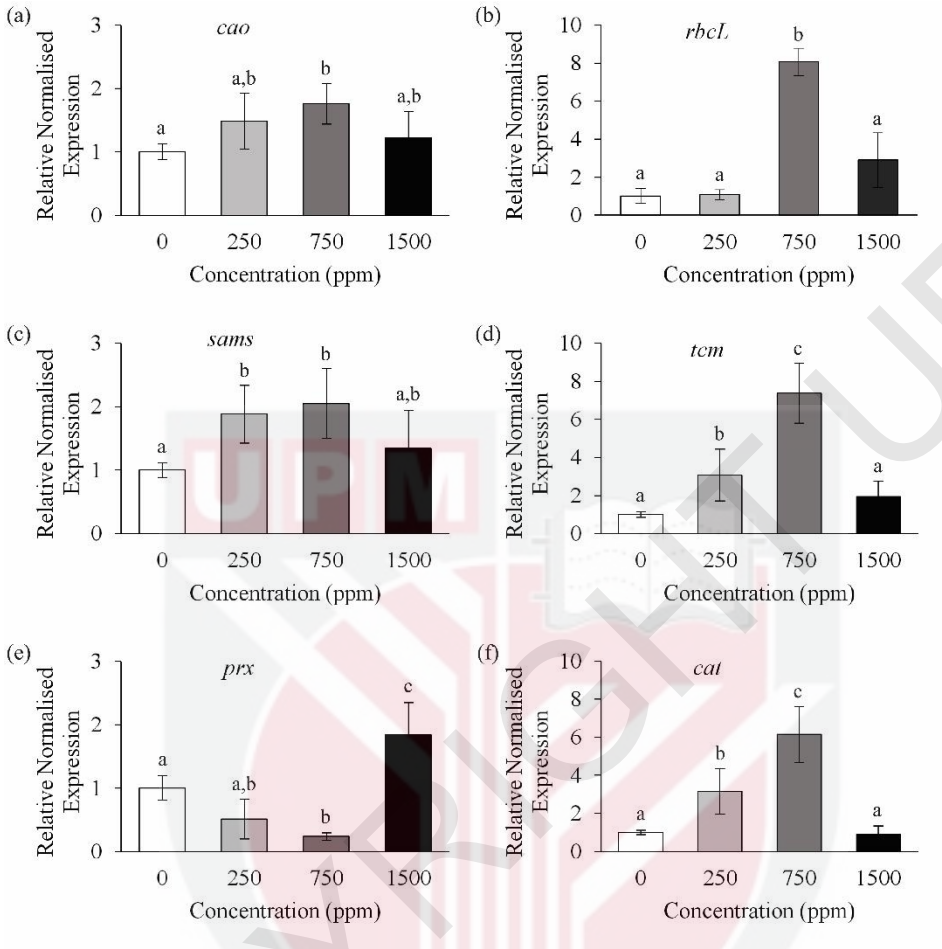


Figure 4: Gene expression analysis on banana plantlets treated with different concentrations of λ-carrageenan. (a) Chlorophyllide *a* oxygenase (*CAO*); (b) ribulose-1,5-bisphosphate carboxylase (*RBCL*); (c) S-adenosylmethionine synthetase (*SAMS*); (d) trans-cinnamate 4-monooxygenase (*TCM*); (e) Class III peroxidase (*PRX*); (f) catalase (*CAT*). Results are presented as differential relative transcript abundance. Mean ± SE of three replicates (n=3). Letters (a,b,c) denote significant difference between the control and treatment groups ($p < 0.05$).

included chlorophyllide *a* oxygenase (*CAO*), and ribulose-1,5-bisphosphate carboxylase (*RBCL*) which is related to photosynthesis; S-adenosylmethionine synthetase (*SAMS*) in protein biosynthesis; trans-cinnamate 4-monooxygenase (*TCM*) in production of phenolic compounds, Class III peroxidase (*PRX*) related in reactive oxygen species (ROS) metabolism; and catalase (*CAT*) in eliminating ROS and regulating redox homeostasis. Four target genes (*CAO*, *RBCL*, *SAMS*, and *TCM*) were found to be upregulated in all λ-carrageenan treatments as compared to the control (Figure 4a-d). Banana plantlets treated with optimum treatment recorded the highest expression levels of *CAO* (1.76-fold) and *RBCL* (8.07-fold). As reported previously, overexpression of *AtCAO* resulted in modulation of the chlorophyll biosynthesis pathway and increased total chlorophyll in transgenic tobacco (*N. tabacum*) plants, which could improve photosynthesis in plants (Biswal et al., 2012). The *CAO* is responsible for converting chlorophyll *a* to chlorophyll *b*, which absorbs light energy at different wavelengths compared to chlorophyll *a* (Sakuraba et al., 2009). This enables plants to absorb light energy across a broader range of wavelengths. Moreover, chlorophyll *b* also takes part in modulating photosynthetic antenna size, which is crucial for optimising light energy transfer to protect plants from photodamage (Tanaka et al., 2001). The elevation of *RBCL* gene expression was also found to increase photosynthetic performance in *Sinapis alba* L., *Robinia pseudoacacia* L., and *Lupinus luteus* L. upon application of vermicompost (Jaskulak et al., 2018). As *CAO* and *RBCL* genes are involved in chlorophyll biosynthesis and carbon fixation, high expression of these genes may cause improvement in photosynthetic activity in λ-carrageenan treatment.

Similarly, in this study, the most upregulation of *SAMS* (2.05-fold) and *TCM* (7.38-fold) genes were also detected in optimum λ-carrageenan treatment. The *SAMS* gene is essential for the biosynthesis of S-adenosylmethionine, which is a methyl group donor for transmethylation reactions. Additionally, the *SAMS* gene takes part in the production of various plant compounds such as methionine, polyamines, nicotinamide, and ethylene (Roje, 2006). In previous studies, growth stunting and abnormality of plant morphology were shown in downregulated *SAMS* transgenic rice and Chinese cabbage (Yu et al., 2012; Li et al., 2011). Nonetheless, *SAMS*-overexpressing Chinese cabbage portrayed rapid plant growth with healthy leaves (Yu et al., 2012). Meanwhile, the *TCM* gene facilitates in production of phenolic compounds such as flavonoids, coumarins, and lignin (Ehlting et al., 2006). It also serves as a key enzyme in the phenylpropanoid pathway, which produces various secondary metabolites involved in plant development and defence. It hydroxylates cinnamic acid to form p-coumaric acid, an intermediate product for the phenylpropanoid pathway (Marchiosi et al., 2020). Overexpression of *TCM* gene increased contents of flavone in *Scutellaria baicalensis* (Kim et al., 2014), pyranocoumarin compound in *Angelica gigas* (Park et al., 2011), and acetosyringone in *N. tabacum* cell suspension (Blount et al., 2002). These reports implied that upregulation of *TCM* expression enhanced the production of phenylpropanoids such as flavonoids and phenolic acids, which take part in various plant developmental processes.

On the other hand, downregulation of Class III *PRX* gene expression was observed in 250 and 750 ppm treatments (Figure 4e). Among all treatments, banana plantlets treated with optimum treatment showed the lowest *PRX* gene expression (4.28-fold). Meanwhile, the expression level of *PRX* gene was upregulated in 1500 ppm treatment

(1.84-fold). The elevated *PRX* gene expression associated with ROS metabolism could stimulate H_2O_2 accumulation and induce oxidative damage and lipid peroxidation in plants (Almagro et al., 2009). Simultaneously, the expression of *CAT* gene was significantly upregulated in 250 (3.16-fold) and 750 ppm (6.15-fold) treatments (Figure 4f). The *CAT* gene is responsible for catalysing the elimination of H_2O_2 and protecting plants from oxidative damage (Huang et al., 2019). In previous reports, ROS-scavenging system was improved in *CAT*-overexpressing transgenic *Arabidopsis* (Chiang et al., 2014), tobacco (Guan et al., 2009), and rice (Moriwaki et al., 2008).

4.3 Biochemical analysis of λ-carrageenan treated banana plantlets

To further support the gene expression analysis, several biochemical contents corresponding to target genes were examined in banana plantlets treated with λ-carrageenan.

4.3.1 Photosynthetic pigments and rubisco activity

The content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*) and total chlorophyll content were shown to increase in all λ-carrageenan treatments (Figure 5a). Banana plantlets treated with 750 ppm treatment showed the highest levels of chlorophyll *a* (11.25 ± 1.13 mg/g FW), chlorophyll *b* (8.27 ± 1.54 mg/g FW), and total chlorophyll (19.52 ± 1.02 mg/g FW). Furthermore, an upregulation of ribulose-1,5-bisphosphate carboxylase/ oxygenase (rubisco) activity was also detected in all λ-carrageenan treatments as compared to the control (Figure 5b). The greatest effect in rubisco activity was observed in 750 ppm λ-carrageenan (0.24 ± 0.017 mmol/min mg protein) which was significantly induced to two folds higher than control (0.07 ± 0.013 mmol/min mg protein).

In this study, the trend observed in total chlorophyll content and rubisco activity was consistent with the expression analysis of photosynthesis-related genes (*CAO* and *RBCL*) via RT-qPCR (Figure 4a, b). Chlorophyll content in plants could affect photosynthetic activity directly. Chlorophyll plays a vital role in photosynthesis, as it absorbs energy from sunlight and converts the light energy into chemical energy (Evans, 2013). The resulting chemical energy is used for carbon fixation and thus sugar molecules are formed. As reported previously, the upregulation of chlorophyll content improved photosynthesis capacity in plants and thus enhanced the growth of cucumber seedlings (Anwar et al., 2020), rapeseed (Lotfi et al., 2015), spinach (Fan et al., 2013), rice (Dhananjaya et al., 2011), and safflower plants (Dordas and Sioulas, 2008). Furthermore, rubisco, a key enzyme in the Calvin cycle, is responsible for carbon fixation and photorespiration (Huang et al., 2015). Improvement in rubisco activity could increase the photosynthetic capacity and formation of carbohydrates in plants. Previous studies claimed that the depletion of enzymes involved in the Calvin cycle resulted in a reduction in photosynthetic capacity and carbohydrate biosynthesis, which ultimately affect plant

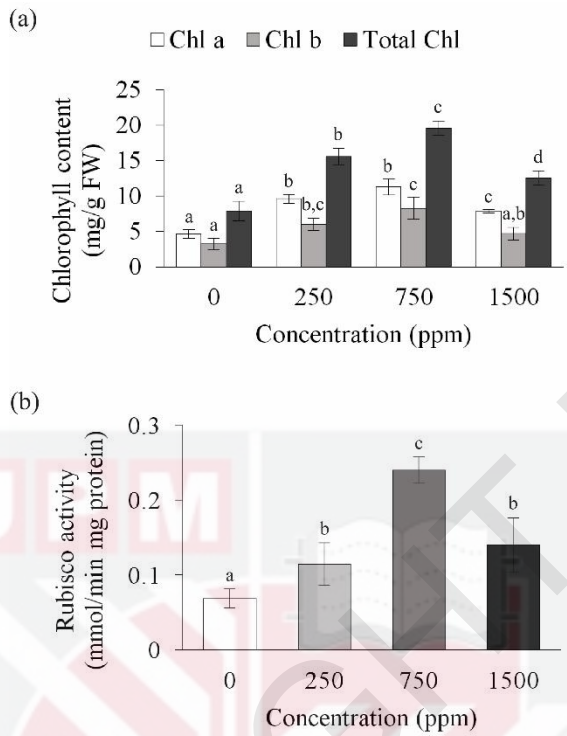


Figure 5: Photosynthetic components in banana plantlets treated with different concentrations of λ-carrageenan. (a) Chlorophyll content; (b) rubisco activity. Results are presented as mean ± SE of three replicates (n=3). Letters (a,b,c,d) denote significant difference between the control and treatment groups ($p < 0.05$).

growth (Harrison et al., 1997; Koßmann et al., 1994). Therefore, photosynthetic capacity in banana plants could be improved through the overall stimulatory effects of chlorophyll contents and rubisco activity in λ-carrageenan treatment, which eventually enhances plant growth. This can be observed from the morphological changes in banana plantlets, where they grew healthier and have bigger leaves upon optimum treatment (Figure 2d).

Nevertheless, banana plantlets treated with 1500 ppm treatment has lower chlorophyll contents and reduced rubisco activity, compared to 750 ppm treatment. This may be attributed to the high manganese level and reduced potassium level in plants. Previous reports revealed that an excess level of manganese negatively affects the photosynthetic apparatus and photosynthesis rate, as well as inhibits chlorophyll synthesis (Messant et al., 2022; Liang et al., 2019). Moreover, photosynthetic activity is impeded by changes in chloroplast morphology and chlorophyll degradation in plants under low potassium conditions (Römheld and Kirkby, 2010; Tian et al., 2008). Therefore, banana plantlets under 1500 ppm treatment showed stunted growth and yellowing of leaves due to decreased photosynthetic activity in plants (Figure 2f).

4.3.2 Total soluble protein and phenolic contents

Quantification of protein content in this study revealed that λ-carrageenan at 750 ppm and 1500 ppm has remarkably increased the content of protein in banana plantlets (Figure 6). As compared to the control (1.76 ± 0.04 μg/g FW), 750 ppm treatment recorded the highest protein content (2.51 ± 0.12 μg/g FW) in banana plantlets. This result was concurrent with the RT-qPCR analysis where optimum treatment recorded the highest expression level of *SAMS* gene (Figure 4c). Plant proteins play essential roles in enzymatic, structural, functional, signalling, and storage functions to facilitate plant growth (Rasheed et al., 2020). Numerous studies demonstrated that there is a positive correlation between protein content and plant growth (Kok et al., 2020; Pereira et al., 2015; Cho et al., 2013; Li et al., 2011; Wullschleger et al., 2006). Previous reports have demonstrated that increase in protein content enhanced protein biosynthesis in plants, and thus improve plant growth in rice (Kok et al., 2021; García et al., 2012; Dhananjaya et al., 2011), rapeseed (Lotfi et al., 2015), and spinach (Fan et al., 2013). This is supported by the observation of optimum λ-carrageenan-treated plants thriving in a healthy and green state (Figure 2d). Enhanced protein content in λ-carrageenan-treated plants could induce various secondary metabolites biosynthesis and regulate metabolic processes, to achieve plant optimum growth.

As shown in Figure 7, 750 ppm treatment exerted a notable elevation in total soluble phenol content (2.75 ± 0.05 mg/g FW) in banana plantlets as compared to control (2.31 ± 0.15 mg/g FW). No significant difference was observed in the 250 ppm and 1500 ppm treatments. The observed trend in total phenol content was coherent with the RT-qPCR analysis of *TCM* gene which is related to the production of phenolic compounds (Figure 4d). Phenolic compounds are crucial in inducing regulatory signal pathways and regulating physiological processes that are necessary for plant growth (Cheynier et al.,

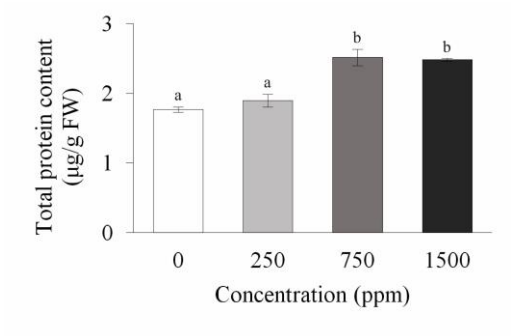


Figure 6: Total protein content in banana plantlets treated with different concentrations of λ-carrageenan. Results are presented as mean ± SE of three replicates (n=3). Letters (a,b) denote significant difference between the control and treatment groups ($p < 0.05$).

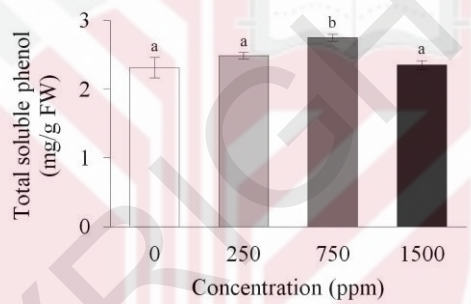


Figure 7: Total phenolic compounds in banana plantlets treated with different concentrations of λ-carrageenan. Results are presented as mean ± SE of three replicates (n=3). Letters (a,b) denote significant difference between the control and treatment groups ($p < 0.05$).

2013; Treutter, 2010). As reported previously, enhanced phenolic compounds are positively correlated with plant growth promotion in spinach (Fan et al., 2013), rice (Dhananjaya et al., 2011), and strawberry callus (López Arnaldos et al., 2001). Apart from this, phenolic compounds also mitigate the harmful effect of stress in plants (Aina et al., 2022). For instance, an increase in phenolic content improved stress tolerance in peppermint (Chiappero et al., 2019), pepper plant (Yildiztekin et al., 2018), and rice (Dhananjaya et al., 2011). Enhanced phenolic compounds not only promote growth in banana plants but also protect them from stress, as evidenced by the vibrant morphology observed in banana plantlets treated with optimum treatment (Figure 2d). In this study, optimum treatment exhibited improved phenolic compounds biosynthesis in banana plantlets, regulating various biological processes and reducing oxidative stress, and this may further enhance growth performance in plants.

4.3.3 Reactive oxygen species and ROS-scavenging enzymes

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in λ-carrageenan treated plantlets were assayed to examine the accumulation of oxidative stress in banana plants. The λ-carrageenan treatments have altered the H_2O_2 and MDA contents in banana plantlets in a concentration-dependent manner (Figure 8a, b). As compared to control (0.0208 ± 0.0019 μM/g FW), the content of H_2O_2 was remarkably decreased in 250 ppm (0.0133 ± 0.0003 μM/g FW) and 750 ppm (0.0128 ± 0.0008 μM/g FW) treatments. Subsequently, the MDA content was also reduced in these two treatments (250 ppm, 0.48 ± 0.05 nmol/g FW; 750 ppm, 0.42 ± 0.06 nmol/g FW). However, 1500 ppm treatment has led to an increment in the contents of H_2O_2 (0.0230 ± 0.0009 μM/g FW) and MDA (0.96 ± 0.10 nmol/g FW) in banana plantlets. The trend observed in H_2O_2 content was coherent with the RT-qPCR expression analysis of Class III *PRX* gene, which is involved in ROS metabolism (Figure 4e). ROS is produced during basic physiological processes such as photosynthesis, photorespiration, and *β*-oxidation of fatty acids (De Gara et al., 2010). ROS in plant cells is needed to be maintained at low concentrations for signalling events in plant growth and defence response (Mhamdi and Van Breusegem, 2018). Nonetheless, a high accumulation of ROS could pose damage and toxicity in plants, leading to oxidative stress, lipid peroxidation, and protein denaturation (You and Chan, 2015). MDA is the end product of lipid peroxidation, which it is triggered by excessive ROS accumulation (Xu et al., 2014). Optimum λ-carrageenan treatment reduced accumulation of H_2O_2 and lipid peroxidation in banana plantlets. Past studies reported that plants treated with plant growth regulators always display reduced levels of H_2O_2 and MDA compared to untreated plants (Esringü et al., 2016; García et al., 2012). The decreased levels of ROS and lipid peroxidation detected in the treated plants suggest that they are protected against oxidative damage, in agreement with the morphological observation of banana plantlets treated with optimum λ-carrageenan (Figure 4d). However, a high concentration of λ-carrageenan induced H_2O_2 production and oxidative stress in banana plantlets. High dosage of λ-carrageenan impose physiological stress and toxicity in plants by disrupting nutrient balance and inhibiting catalase production, leading to the accumulation of H_2O_2 and MDA contents. This can be seen through the observation of plant morphological changes, where the banana plantlets exhibited yellowing of leaves and growth stunting (Figure 2f). In agreement, previous reports suggested that accumulation of high H_2O_2 content and increased lipid peroxidation

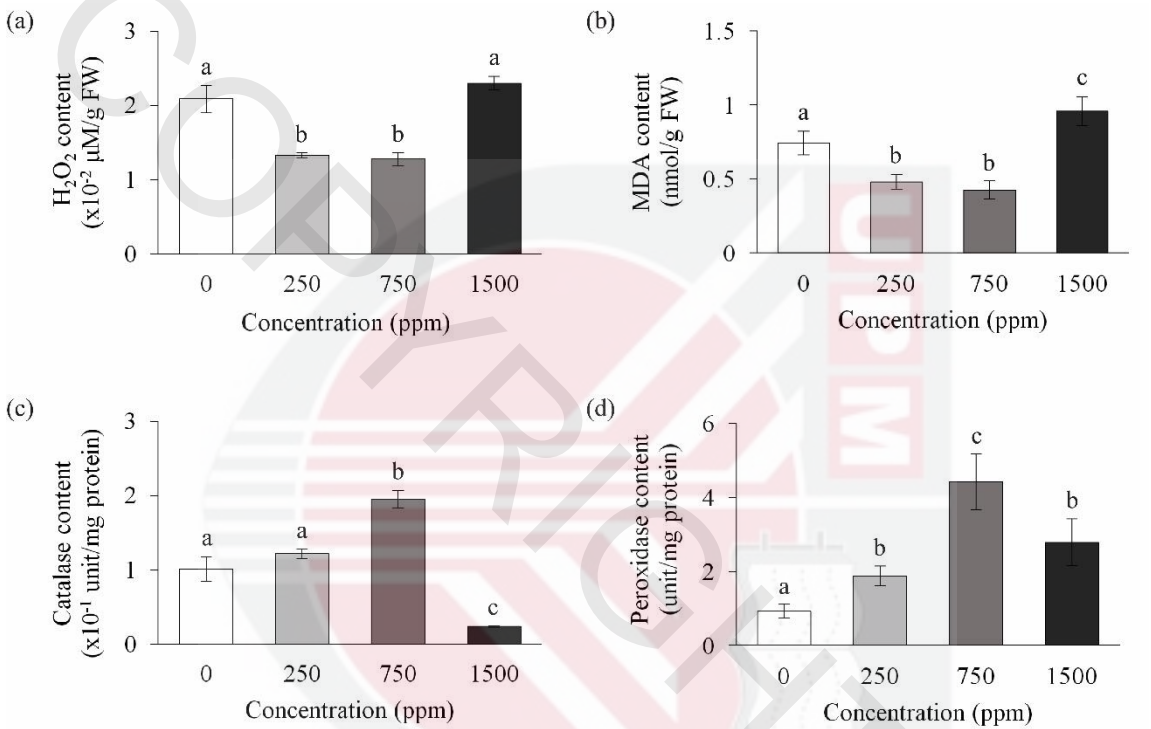


Figure 8: Biochemical analysis related to ROS accumulation and ROS-scavenging enzymes in banana plantlets treated with different concentrations of λ-carrageenan. (a) Hydrogen peroxide content; (b) malondialdyhyde assay; (c) catalase content; (d) total peroxidase content. Results are presented as mean ± SE of three replicates (n=3). Letters (a,b.c) denote significant difference between the control and treatment groups ($p < 0.05$).

hindered plant growth of peppermint (Chiappero et al., 2019), lettuce plants (Ríos et al., 2009), and alfalfa (Wang et al., 2009).

The enzymatic assay was performed to evaluate the effects of different λ-carrageenan concentrations on plantlets catalase and peroxidase activity. In comparison to control (0.101 ± 0.017 unit/mg protein), λ-carrageenan at 750 ppm had a remarkably elevated catalase activity (0.195 ± 0.012 unit/mg protein) in banana plantlets, meanwhile, 1500 ppm treatment recorded a significant reduction (0.024 ± 0.001 unit/mg protein) in catalase activity (Figure 8c). In addition, all λ-carrageenan treatments exhibited an increment in peroxidase activity (Figure 8d). A significant elevation of peroxidase activity was observed in banana plantlets treated with 750 ppm (4.42 ± 0.76 units/mg protein) and 1500 ppm (2.78 ± 0.63 units/mg protein) treatments as compared to control (0.93 ± 0.19 unit/mg protein). No notable effect in catalase and peroxidase activity was detected in the 250 ppm treatment. The observed trend in catalase content was consistent with the *CAT* gene expression analysis (Figure 4f). ROS-scavenging enzymes such as catalase and peroxidase play vital roles in eliminating ROS and maintaining cell homeostasis in plants (Chapman et al., 2019; Huang et al., 2019; You and Chan, 2015). Previous reports claimed that upregulation of catalase and peroxidase effectively reduced H_2O_2 generation and lipid peroxidation in alfalfa upon NaCl treatment (Wang et al., 2009) and strawberries upon blue light treatment (Xu et al., 2014). Therefore, optimum treatment improved the production of ROS-scavenging enzymes to alleviate H_2O_2 accumulation and lipid peroxidation in plants. Regardless, high-concentration treatment recorded a decline in catalase content and an upregulation in peroxidase content in banana plantlets. Reduction in catalase content may result in a further build-up of H_2O_2 content in plants, and this could not be completely offset by the upregulated peroxidase activity. As reported previously, oxidative stress was detected in rice leaves treated with excess iron although it showed no changes in catalase activity but increased activity of ascorbate peroxidase (Fang et al., 2001). Therefore, treatment with higher λ-carrageenan induces stress in banana plants via accumulation of H_2O_2, lipid peroxidation, and reduced catalase activity.

4.4 Nutrient ion contents in banana plantlets under λ-carrageenan treatment

Nutrient analysis was carried out to assess the effects of λ-carrageenan on nutrient uptake in banana plants. The tested nutrient ions included nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) which are categorized as macronutrients; additionally, iron (Fe), manganese (Mn), and zinc (Zn) which are categorized as micronutrients. The nutrient ion analysis revealed that λ-carrageenan at 750 ppm concentration exerted a remarkable increment in the contents of macronutrients on banana plantlets as compared to the control (Table 3). Contents of N (0.389 ± 0.01 g/100 g), P (432 ± 11.36 mg/kg), K (343 ± 11.27 mg/100 g), and Mg (74.1 ± 1.24 mg/100 g) were notably increased in 750 ppm treatment. Similarly, a significant increment in P (574 ± 10.82 mg/kg), Ca (94.7 ± 1.45 mg/100 g), Mg (62.6 ± 2.29 mg/100 g), and Mn (77.1 ± 1.75 mg/kg) was observed in 1500 ppm treatment. Nonetheless, 750 ppm treatment has led to a notable reduction in the content of Mn (11.0 ± 1.28 mg/kg),

Table 4: Nutrient ion analysis on banana plantlets treated with different concentrations of λ-carrageenan.

	Nutrient Content							
Concentration (ppm)	**N (g/100 g)**	**P (mg/kg)**	**K (mg/100 g)**	**Ca (mg/100 g)**	**Mg (mg/100 g)**	**Fe (mg/kg)**	**Mn (mg/kg)**	**Zn (mg/kg)**
0	0.365 ± 0.01	414 ± 6.24	315 ± 7.21	48.1 ± 7.15	40.1 ± 2.69	6.47 ± 0.43	60.9 ± 1.99	2.17 ± 0.62
750	0.389 ± 0.01*	432 ± 11.36*	343 ± 11.27*	36.3 ± 3.56	74.1 ± 1.24*	5.22 ± 0.70	11.0 ± 1.28*	1.08 ± 0.35
1500	0.337 ± 0.02	574 ± 10.82*	245 ± 14.93*	94.7 ± 1.45*	62.6 ± 2.29*	6.39 ± 0.10	77.1 ± 1.75*	1.31 ± 0.27

Means ± SE of three replicates (n=3) followed by * in a row which indicates statistical significance between control and λ-carrageenan-treated samples ($p < 0.05$).

whereas 1500 ppm λ-carrageenan significantly reduced K content (245 ± 14.93 mg/100 g) in treated banana plantlets.

The plant absorbs nutrients from the soil and transports them to other parts for various cellular processes (Maathuis and Diatloff, 2013). Plant nutrients are divided into two major groups, namely macronutrients which require in high amounts, and micronutrients only needed in trace amounts. In this study, optimum treatment increased most of the macronutrients (N, P, K, and Mg), but decreased Mn content in banana plantlets. The enhancement of nutrient uptake in banana plantlets was believed to be caused by root structure improvements in optimum λ-carrageenan (Figure 2d). Nitrogen, phosphorus, potassium, and magnesium are essential minerals that are required for plant metabolism such as photosynthesis, protein synthesis, energy metabolism, and enzymatic activity. For instance, lacking nitrogen caused growth stunting in maize (Mu et al., 2016), while a deficiency of potassium decreased leaf area in soybean and cotton (Pettigrew, 2008). Notwithstanding, a decrease in manganese uptake could be due to the high concentration of phosphorus in plants. Pedas et al. (2011) reported that the application of phosphorus had decreased the manganese uptake in barley. The high nutrient contents observed in banana plantlets resulted in enhanced plant growth, as shown in Figure 2d, where the treated plantlets grew vibrantly and healthily. Taken together, optimum λ-carrageenan enhanced macronutrient absorption in banana plantlets, which subsequently induces various metabolic processes and improves plant growth.

On the other hand, a high concentration of λ-carrageenan increased the contents of phosphorus, calcium, magnesium, and manganese, as well as reduced potassium content in banana plantlets. High calcium uptake may inhibit plant growth and impose toxicity on plants (Wei et al., 2018). Similarly, excess manganese content can negatively affect numerous metabolic processes and trigger oxidative stress in plants, imposing toxicity and eventually hindering plant growth (Millaleo et al., 2010). Nonetheless, a reduction in potassium uptake cause chlorosis and growth retardation in plants (Pettigrew, 2008). Increased micronutrient contents could contribute to the high ROS accumulation and oxidative stress in banana plantlets treated with a high concentration of λ-carrageenan as shown in previous Section 4.3.3, and thus the treated banana plantlets exhibited growth stunting and leaf yellowing (Figure 2f). In short, high λ-carrageenan treatment increased micronutrient uptake, resulting in the accumulation of stress and toxicity, hence negatively affecting the growth of plants.

4.5 Metabolomics analysis of banana plantlets treated with λ-carrageenan

4.5.1 Metabolite identification by 1D and 2D NMR spectral analysis

The difference in metabolite composition in banana plantlets (n = 4) upon optimum λ-carrageenan treatment was achieved using metabolomics profiling via NMR. The detailed analysis of the metabolite profile was conducted by utilising a combination of ^{1}H NMR, as well as 2D NMR such as JRES and HSQC. The 2D JRES and HSQC NMR

spectroscopy was employed to ease confirmation on the identity of the metabolites particularly of overlapping signals observed in the 1D NMR spectra, which hamper the peak assignment and metabolites identification.

Figure 9 shows the representative of ^{1}H NMR spectra for leaf extracts from control and optimum λ-carrageenan treatment. Visual inspection of the overlapped spectra portrayed the differential metabolic intensity, especially in the region of 3.0 to 5.0 ppm. Another visible varied region was 1.0 to 2.0 ppm, which mainly represented the aliphatic group. Metabolite identifications were done by comparing 1D NMR chemical shifts with previous literature and matching with publicly available metabolomics databases like the Human Metabolome Database (HMDB, http://www.hmdb.ca). The HMDB was used in this study due to its extensive collection of metabolite data (Wishart et al., 2022).

However, there is still a very limited reference metabolome and spectral data available in plant metabolome databases (Shi et al., 2024). Verification was then carried out through 2D J-resolved and two-dimensional HSQC analysis. A total of 26 compounds (Table 4) from several classes were successfully identified from the metabolite profiles. The identified compounds include carbohydrates namely glycerol, glucose 6-phosphate, fructose, glucosamine 6-phosphate, sucrose, *α*-glucose, and *β*-glucose, amino acids and derivatives such as glycine, valine, isoleucine, lysine, glutamate, *γ*-aminobutyrate, and glycine betaine, lipids and lipid-like molecules which are lupeol, *α*-linolenic acid, 3-hydroxy-3-methylglutaryl-CoA, and phosphatidylcholine, as well as ethanol, acetate, acetone, lutein, catechin, allantoin, choline, and acetylcholine.

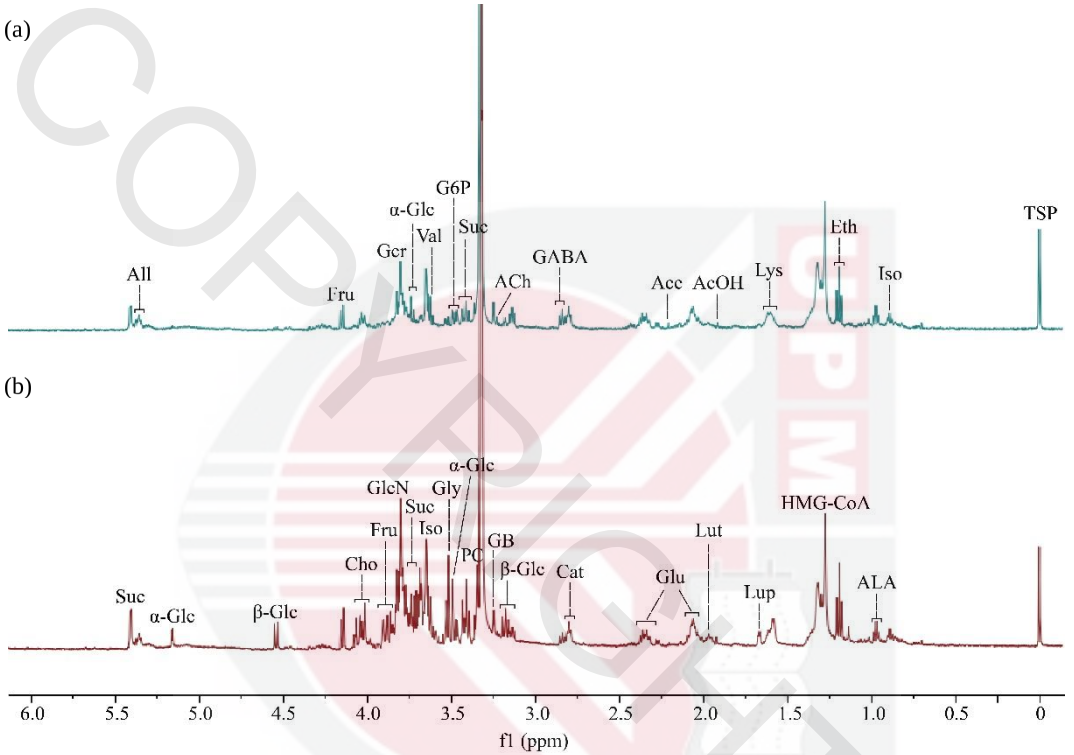


Figure 9: The representative of ^{1}H NMR spectra of banana plantlets for (a) control and (b) optimum λ-carrageenan treatment. Ace, acetone; ACh, acetylcholine; AcOH, acetate; ALA, *α*-linolenic acid; All, allantoin; Cat, catechin; Cho, choline; Eth, ethanol; Fru, fructose; *α*-Glc, *α*-glucose; *β*-Glc, *β*-glucose; G6P, glucose 6-phosphate; GABA, *γ*-aminobutyrate; GB, glycine betaine; GlcN, glucosamine 6-phosphate; Gcr, glycerol; Glu, glutamate; Gly, glycine; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; Iso, isoleucine; Lup, lupeol; Lut, lutein; Lys, lysine; PC, phosphatidylcholine; Suc, sucrose; Val, valine; TSP, trimethylsilyl propanoic acid.

Table 5: List of putative compounds and their ^{1}H-chemical shifts (ppm) with coupling pattern*, coupling constant (Hz) and HSQC (2D) correlation.

No	Putative metabolite	Chemical shift (ppm), multiplicity, J (Hz)	HSQC (^{13}C)
1	Isoleucine	0.89 (t, 6.8) 3.68 (d, 6.0)	16.21
2	α-linolenic acid	0.97 (t, 7.5)	16.60
3	Ethanol	1.19 (t, 7.1) 3.64 (q)	20.12 65.24
4	3-hydroxy-3-methylglutaryl-CoA	1.3 (s)	
5	Lysine	1.58 (m)	27.74
6	Lupeol	1.66 (s)	
7	Acetate	1.91 (s)	
8	Lutein	1.97 (s)	
9	Glutamate	2.05 (m) 2.35 (dt)	29.88 36.92
10	Acetone	2.21 (s)	
11	Catechin	2.82 (m)	
12	γ-aminobutyrate	2.84 (t, 7.7)	
13	β-glucose	3.16 (m) 4.54 (d, 7.8)	99.62
14	Acetylcholine	3.22 (s)	
15	Glycine betaine	3.24 (s)	
16	Phosphatidylcholine	3.33 (s)	

Table 6: Continued

17	Sucrose	3.42 (m) 3.75 (m) 5.41 (d, 3.8)	65.82 72.86 75.20 95.32
18	Glucose 6-phosphate	3.48 (dd, 9.8, 3.8)	74.61
19	α-glucose	3.51 (s) 3.75 (m) 5.16 (d, 3.7)	65.82 74.61 76.18
20	Glycine	3.53 (s)	66.22
21	Valine	3.60 (d, 6.9)	
22	Glycerol	3.8 (d, 5.8)	63.87 64.07 74.61
23	Glucosamine 6-phosphate	3.87 (dd; 1.8; 12.0)	64.26
24	Choline	4.03 (m)	
25	Fructose	3.65 (d, 2.5) 4.15 (d, 8.5)	80.67
26	Allantoin	5.36 (d, 8.7)	

*s: singlet, d: doublet, t: triplet, dd: doublet of doublet, dt: doublet of triplets, q: quartet, m: multiplet.

4.5.2 Multivariate analysis on ^{1}H NMR data

Metabolite changes upon optimum λ-carrageenan treatment were analysed by the unsupervised PCA. The PCA was used to determine the similarities and differences in metabolic profiles between the samples. The score plot revealed that the treatment group can be discriminated from the control (Figure 10a). The goodness of fit and predictability of the model showed $R^2X = 0.866$ and $Q^2 = 0.569$. A large Q^2 value, which is more than 0.5 but smaller than R^2 value, implied that the generated model has good predictability (Eriksson et al., 2006). Additionally, the variation between R^2 and Q^2 is preferable to be in the range of 0.2 to 0.3. However, the Q^2 value could be considered low due to some replicates of the treatment and control groups that are non-discriminated due to their close position at the middle of the plot. The score plot indicates that 47.6% of the separation is attributed to PC1, while 23.8% is attributed to PC2, accounting for a total of 71% of the variance. The PCA loading plot was used to determine the compounds responsible for the discrimination of groups (Figure 10b). Acetone, phosphatidylcholine, glycerol, α-glucose, β-glucose, glucose 6-phosphate, glucosamine 6-phosphate, sucrose, fructose, glycine, valine, isoleucine, choline, and lupeol, which located on the negative side of PC1, were more prominent in the treatment group. Meanwhile, the compounds located in the positive side of PC1 are those corresponding to the control group, included α-linolenic acid, ethanol, 3-hydroxy-3-methylglutaryl-CoA, acetylcholine, glycine betaine, lysine, glutamate, lutein, allantoin, acetate, catechin, and γ-aminobutyrate.

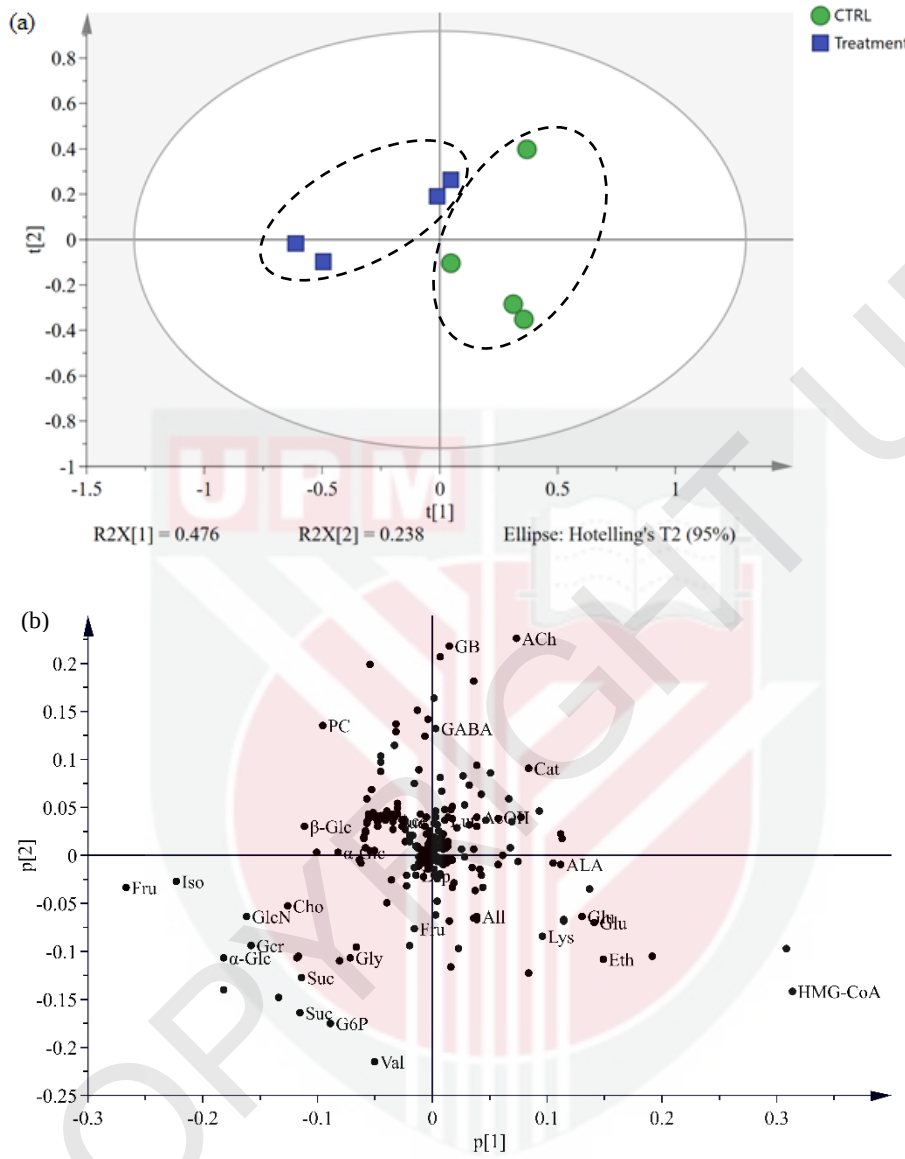
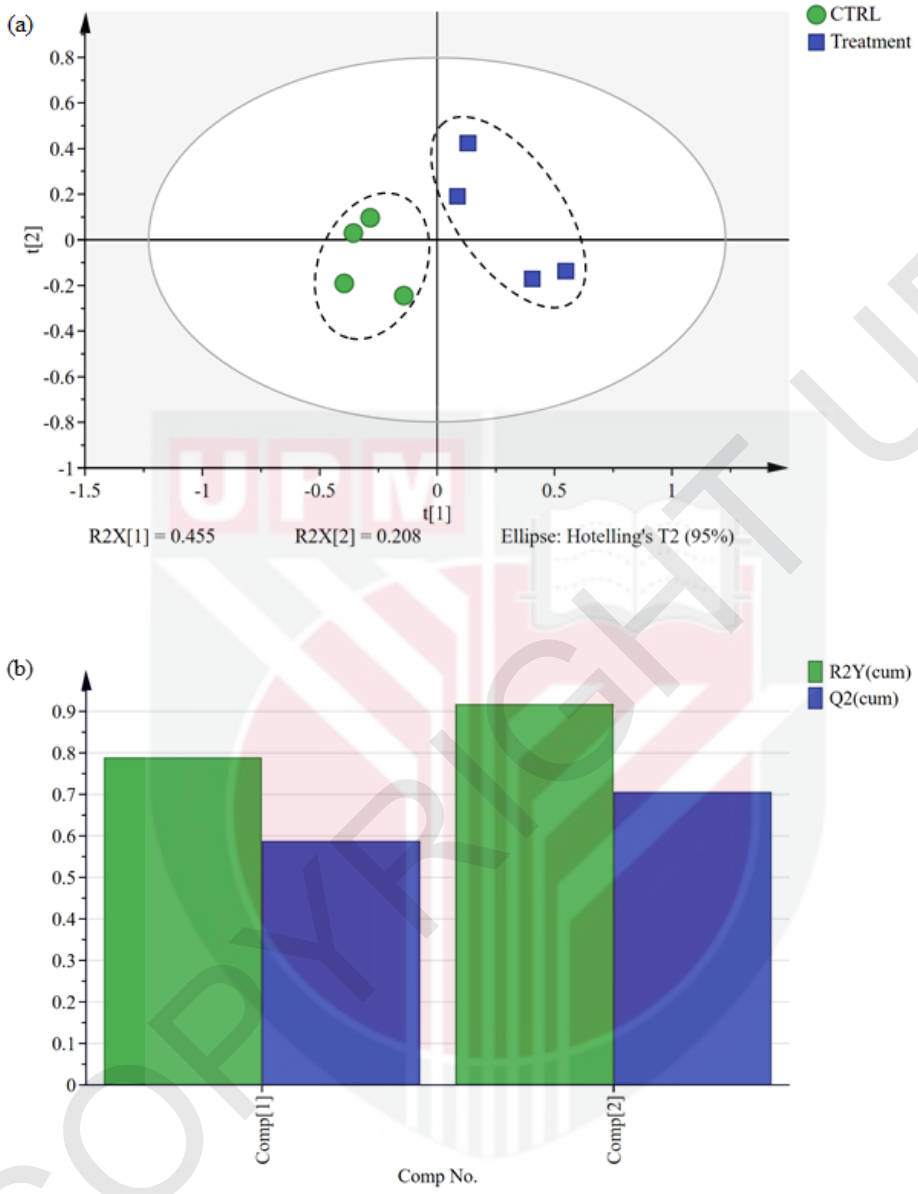


Figure 10: PCA score plot (a) and loading scatter plot (b) of banana plantlets ^{1}H NMR spectral data for control and optimum λ-carrageenan treatment. Ace, acetone; ACh , acetylcholine; AcOH, acetate; ALA, *α*-linolenic acid; All, allantoin; Cat, catechin; Cho, choline; Eth, ethanol; Fru, fructose; *α*-Glc, *α*-glucose; *β*-Glc, *β*-glucose; G6P, glucose 6-phosphate; GABA, *γ*-aminobutyrate; GB, glycine betaine; GlcN, glucosamine 6-phosphate; Gcr, glycerol; Glu, glutamate; Gly, glycine; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; Iso, isoleucine; Lup, lupeol; Lut, lutein; Lys, lysine; PC, phosphatidylcholine; Suc, sucrose; Val, valine.

To further evaluate the results, a supervised PLS-DA model was conducted. The PLS-DA score plot showed control and treatment groups were distinctly separated by PC1 with the control in the left quadrant and the treatment group in the right quadrant of the model (Figure 11a). PC1 is responsible for the separation of treatment group from the control group as it contributed 45.5% of the separation, while PC2 contributed 20.8%, constituting a total of 66% of the variance. As shown in Figure 11b, the goodness of fit and predictability of the model showed two components with acceptable values of R^2Y (0.917) and Q^2 (0.706), where the difference between R^2 and Q^2 is smaller than 0.3. The permutation test demonstrated the y-intercept of Q^2 below zero (-0.14), confirming the minimum validity of the constructed model as per SIMCA's criteria and indicating that the model is not overfitting (Figure 11c). The variable importance for the projection (VIP) plot portrayed the important metabolites contributing to the class separation (Figure 11d). Metabolites with a VIP value of more than 0.7 with error bars not crossing zero were considered significant (Kim et al., 2011). A total of 13 metabolites was found to be significantly influenced in optimum λ-carrageenan treatment, namely *α*-linolenic acid, choline, ethanol, fructose, *α*-glucose, *β*-glucose, glucose 6-phosphate, glucosamine 6-phosphate, glycerol, glycine, 3-hydroxy-3-methylglutaryl-CoA, isoleucine, and phosphatidylcholine (Table 5).



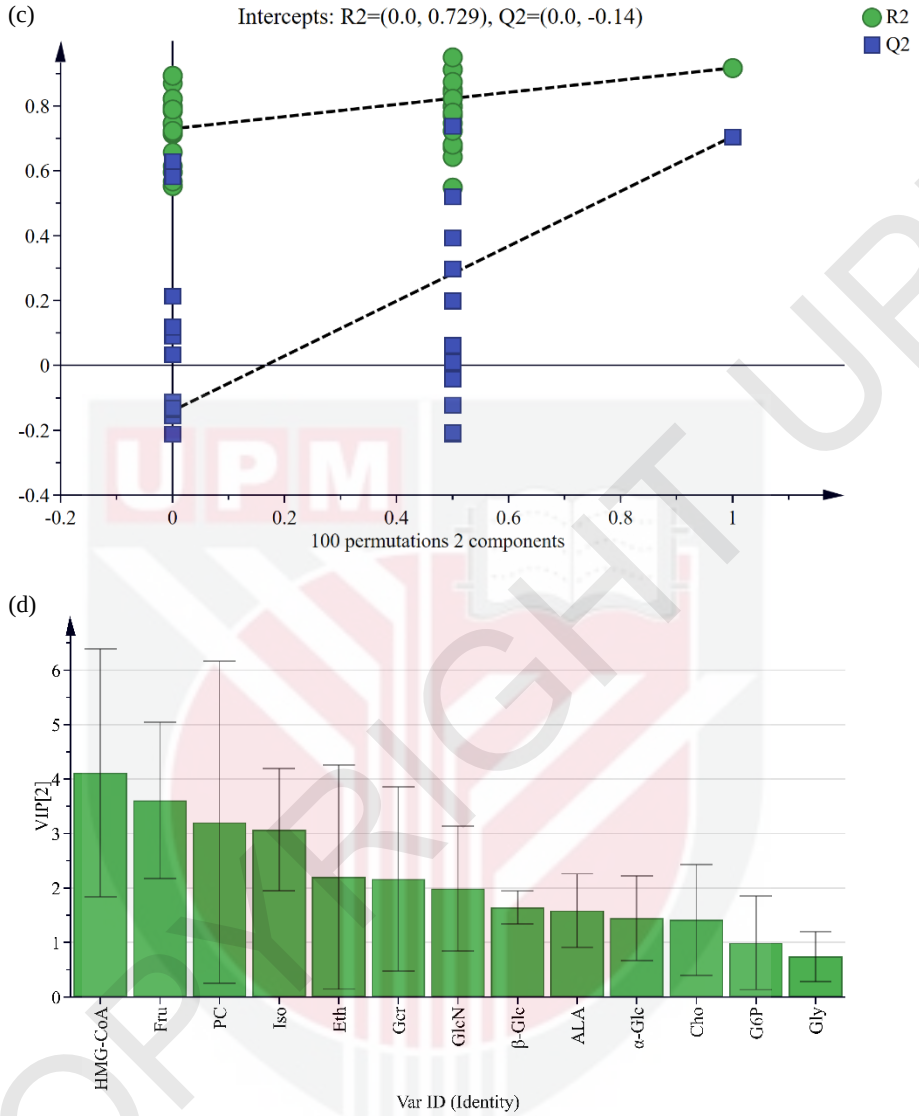


Figure 11: PLS-DA score plot (a), summary of fit generated (b), permutation test (c), and VIP plot (d) for banana plantlets of control and optimum λ-carrageenan treatment. ALA, *α*-linolenic acid; Cho, choline; Eth, ethanol; Fru, fructose; *α*-Glc, *α*-glucose; *β*-Glc, *β*-glucose; G6P, glucose 6-phosphate; GlcN, glucosamine 6-phosphate; Gcr, glycerol; Gly, glycine; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; Iso, isoleucine; PC, phosphatidylcholine.

Table 7: ^{1}H NMR signal assignment of λ-carrageenan treated banana plantlets against control, their VIP and fold change values along with associated metabolic pathways.

Metabolites	Chemical shift (ppm)	VIP value	Fold change (Treatment vs. control	Metabolic pathway
Upregulated				
Phosphatidylcholine	3.34	3.21	0.32	Lipid metabolism
Glucose 6-phosphate	3.48	1.99	0.03	Carbohydrate metabolism
Glycine	3.54	0.74	0.08	Glycine, serine, and threonine metabolism
Fructose	3.66	3.61	0.92	Carbohydrate metabolism
Isoleucine	3.70	3.07	0.80	Valine, leucine, and isoleucine metabolism
Glycerol	3.82	2.16	0.24	Lipid metabolism
Glucosamine 6-phosphate	3.86	1.99	0.69	Carbohydrate metabolism
Choline	4.06	1.41	1.70	Lipid metabolism
β-glucose	4.54	1.64	0.14	Carbohydrate metabolism
α-glucose	5.14	1.44	2.76	Carbohydrate metabolism
Downregulated				
α-linolenic acid	0.98	1.59	-0.28	Lipid metabolism
Ethanol	1.18	2.20	-0.28	Glycolysis
3-hydroxy-3-methylglutaryl-CoA	1.3	4.12	-0.26	Terpenoids biosynthesis

4.5.3 Associated metabolic pathway involved in banana plantlets treated with optimum λ-carrageenan treatment

The identified metabolites with VIP values greater than 0.7, their fold change values, and the associated metabolic pathways are listed in Table 5. Based on the fold change values obtained between the intensity differences of the treatment and control groups, ten metabolites were notably upregulated in optimum treatment, namely phosphatidylcholine, glucose 6-phosphate, glycine, fructose, isoleucine, glycerol, glucosamine 6-phosphate, choline, *β*-glucose, and *α*-glucose. On the other hand, a significant downregulation of three metabolites was also detected in the optimum treatment, including *α*-linolenic acid, ethanol, and 3-hydroxy-3-methylglutaryl-CoA. The associated metabolic pathways were suggested by the Kyoto Encyclopedia of Genes and Genomes (KEGG) in which the upregulated metabolites were mainly involved in carbohydrate metabolism, amino acid biosynthesis, and lipid metabolism. While the downregulated metabolites were associated with lipid metabolism, glycolysis, amino acid metabolism as well as biosynthesis of terpenoids. Figure 12 summarises the associated metabolic pathway involved in banana plantlets upon optimum treatment.

4.5.3.1 Optimum λ-carrageenan effect on carbohydrate metabolism

Five metabolites, include glucosamine 6-phosphate, glucose 6-phosphate, fructose, *β*-glucose, and *α*-glucose, were associated with carbohydrate metabolism were remarkably upregulated in the optimum treatment group (Table 5). Meanwhile, ethanol, which is associated with glycolysis, was found to be downregulated. Carbohydrate metabolism plays a crucial role in plants by serving as energy source, providing structural support, and participating in various physiological processes (Slewinski and Braun, 2010). Improvement of carbohydrate metabolism was believed to be due to the enhancement of photosynthetic capacity in banana plantlets as shown in the previous Section 4.3.1, which is in agreement with other reported studies (Kok et al, 2021; Kang et al., 2014). Sugars and other carbohydrates produced from photosynthesis are broken down into simple sugars and used to generate energy through glycolysis and the Krebs cycle. The energy was generated in the form of ATP and used in various cellular processes to regulate plant growth and development (Zhong et al., 2016). In brief, glucose is converted into glucose 6-phosphate and initiates glycolysis. Meanwhile, glucosamine 6-phosphate is converted into fructose 6-phosphate, a derivative of fructose, which is subsequently converted into glucose 6-phosphate. Besides, glucosamine 6-phosphate, as an intermediate in the biosynthesis of alanine, aspartate, and glutamate, is also involved in amino sugar and nucleotide sugar metabolism. Some previous reports demonstrated that the enhancement of carbohydrate metabolism has resulted in plant growth promotion in rice (Kok et al., 2021), and mustard plants (Kang et al., 2014). Therefore, upregulation of these metabolites suggested that optimum λ-carrageenan treatment could induce carbohydrate metabolism, which in turn promotes plant growth in banana plants, as shown in Figure 2d.

Nevertheless, ethanol is synthesised from acetaldehyde under anaerobic conditions (Maricle et al., 2014). Pyruvate produced from glycolysis is converted into acetaldehyde

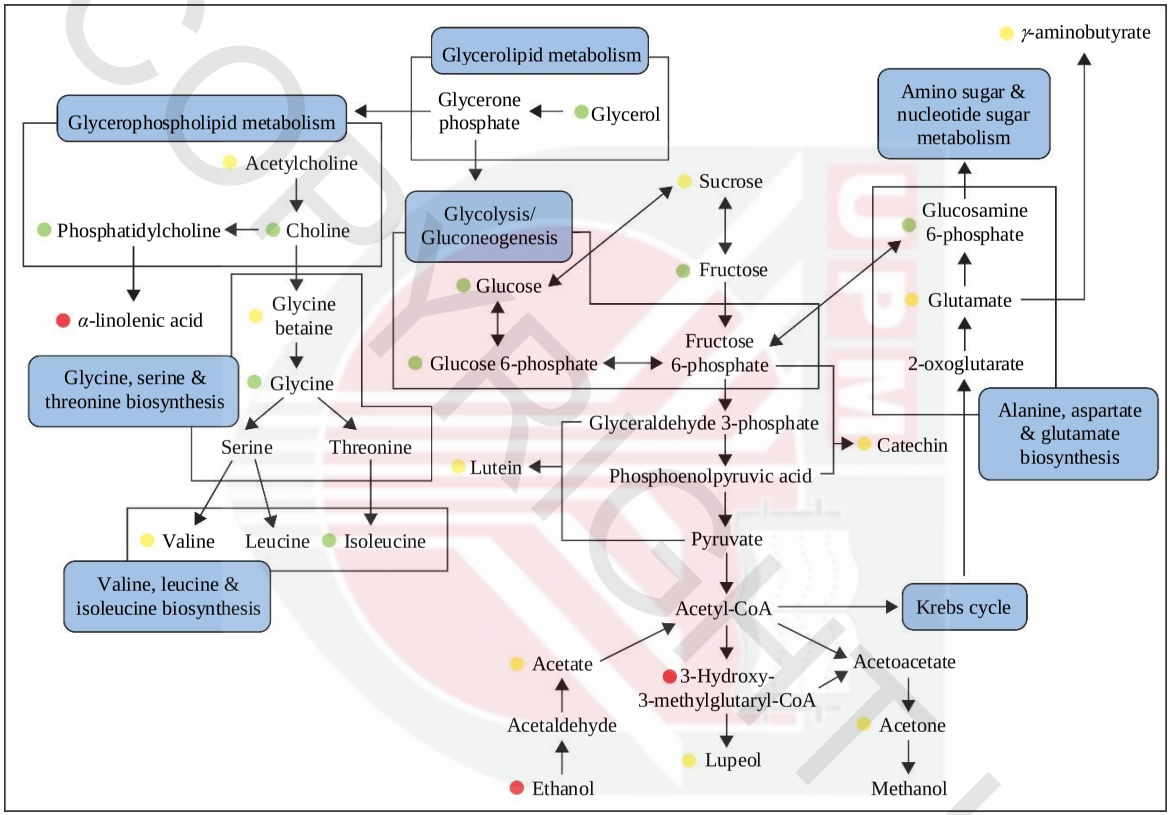


Figure 12: Schematic diagram of proposed metabolic pathways involved in optimum λ-carrageenan treatment. Green and red circles represent upregulated and downregulated metabolites, respectively, in optimum treatment compared to the control. Yellow circle represents metabolites detected during analysis, while blue box represents metabolic pathway involved.

and is further converted into ethanol. Decreased ethanol in optimum treatment may be attributed to the enhanced carbohydrate metabolism in banana plantlets. The pyruvates are directed to be transformed into acetyl-CoA and initiate the Krebs cycle to produce energy aerobically. Moreover, the accumulation of ethanol and acetaldehyde in plants has been documented to induce toxicity in plant cells, leading to the disruption of lipid-protein bonds and subsequent denaturation of proteins (Maricle et al., 2014; Mitcham and McDonald, 1993).

4.5.3.2 Optimum λ-carrageenan effect on amino acid metabolism

Similarly, amino acids such as glycine and isoleucine were significantly upregulated in optimum treatment (Table 5). Amino acids serve as the building blocks for protein synthesis and precursors for many primary and secondary metabolites, which play important roles in plant growth and development (Less and Galili, 2008). As reported previously, the induced production of amino acids was positively correlated with the enhancement of plant growth in mustard plants (Kang et al., 2014) and cucumber plants (Kang et al., 2012). Upregulation of amino acids biosynthesis in banana plants could enhance protein biosynthesis as well as production of primary and secondary metabolites, and hence improve growth of banana plants. This finding coincides with the high content of proteins detected in optimum treatment (Figure 6) and thus leads to the healthy plant morphology in banana plantlets (Figure 2d).

4.5.3.3 Optimum λ-carrageenan effect on lipid metabolism

Three metabolites namely phosphatidylcholine, glycerol, and choline, associated with lipid metabolism were identified to be notably upregulated in optimum treatment, whereas *α*-linolenic acid was significantly downregulated in the optimum treatment group (Table 5). In plants, lipids are synthesised for membrane structure, energy storage, regulatory signalling, and protection (Lavell and Benning, 2019). Glycerol, the structural backbone of lipid molecules, participates in the formation of simple and complex lipids, which is vital for living organisms. In addition, glycerol also takes part in various metabolic processes, for example, photosynthesis and glycolysis (Gull and Pasek, 2021). Choline plays several important functions in plants such as contributing to membrane integrity, antioxidant activity, signalling, and growth regulation. It is a precursor for the synthesis of phosphatidylcholine, which is a major component of plant cell membranes. Phosphatidylcholine helps maintain the integrity and fluidity of cell membranes, thereby ensuring proper cellular structure and function. Moreover, choline is a precursor for glycine betaine biosynthesis, where glycine betaine helps protect plant cells against oxidative damage. Gou et al. (2015) claimed that accumulation of choline in maize enhances glycine betaine metabolism and hence induces stress resistance. Upregulation of glycerol, choline and phosphatidylcholine in banana plantlets could enhance plant growth and protect them from oxidative stress. This result is coherent with the reduced ROS accumulation and lipid peroxidation as shown in previous Section 4.3.3, hence banana plantlets treated with optimum treatment exhibited vibrant morphology (Figure 2d).

On the other hand, α-linolenic acid is an unsaturated fatty acid that is strongly linked to the antioxidant function and biosynthesis of jasmonic acid (JA) in plants. Numerous studies claimed that JA biosynthesis is deeply related to abiotic and biotic stresses (Wang et al., 2020; Ruan et al., 2019). Downregulation of α-linolenic acid in optimum treatment may be due to the reduced ROS accumulation and lipid peroxidation in banana plantlets (Section 4.3.3). For instance, upregulated α-linolenic acid was detected in maize under drought stress, compared to the unstressed plants (Zi et al., 2022).

4.5.3.4 Other metabolism

Downregulation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), which involved in the mevalonate pathway for terpenoid biosynthesis, was observed in optimum treatment (Table 5). The HMG-CoA is converted into mevalonate by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and is further converted into isopentenyl diphosphate, a building block of terpenoids (Tholl, 2015). Past studies had demonstrated that overexpression in HMGR could enhance terpenoid content in plants (Wei et al., 2019; Xu et al., 2012; Enfissi et al., 2005). Terpenoids play an important role in alleviating the effects of oxidative stress in plants. The HMG-CoA is directed to produce terpenoids in plants, thus enhancing protection against oxidative stress. This finding aligned with the low accumulation in ROS and lipid peroxidation in the treated group compared to control (Section 4.3.3).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, the effects of λ-carrageenan on the growth of *Musa acuminata* cv. Berangan was successfully investigated. The optimum concentration for banana plants was identified at 750 ppm via concentration study, which gave the best results on the growth performance of banana plantlets. Furthermore, the effects of λ-carrageenan on banana plant growth and defence response were successfully assessed via gene expression analysis, biochemical assays, and nutrient ion analysis. Upon treatment with optimum λ-carrageenan, expression levels of growth-related genes (*CAO*, *RBCL*, *SAMS*, and *TCM*) and ROS scavenging-related gene (*CAT*) were upregulated, meanwhile, the expression level of *PRX* gene involved in ROS metabolism was downregulated. Results obtained from biochemical analysis were concurrent with the gene expression analysis, where increment of chlorophyll content, rubisco activity, protein content, and content of phenolic compounds were observed in optimum treatment. Stimulation of ROS-scavenging enzymes, catalase, and peroxidase, in optimum treatment, were coupled with reduction of hydrogen peroxide and malondialdehyde accumulation, which leads to maintenance of redox homeostasis and enhanced defence responses. Further nutrient analysis of the banana plants displayed that most of the macronutrient uptake was elevated in optimum treatment. In addition, metabolomics profiling analysis via NMR revealed the upregulation of metabolites associated with carbohydrate metabolism, lipid metabolism, and amino acid biosynthesis in optimum λ-carrageenan. Taken together, the probable growth-promoting mechanism of λ-carrageenan in banana plants was identified. These results suggest that the corporation of λ-carrageenan at optimum concentration improved growth in banana plants via upregulation of several cell metabolism, enhancement of macronutrient uptake, and maintenance of cellular homeostasis (Figure 13).

In contrast, inhibition of plant growth was observed in banana plants treated with higher concentrations of λ-carrageenan. A concentration of 1500 ppm was identified as an extreme treatment. Stunted growth and yellowing of leaves were detected in extreme treatment. Accumulation of hydrogen peroxide and malondialdehyde was observed together with the downregulation of catalase in banana plants treated with extreme concentration. Moreover, nutrient analysis revealed that high uptake of micronutrients in extreme treatment, which further contributed to oxidative stress accumulation in plants. Therefore, extreme concentrations of λ-carrageenan induced stress and inhibited plant growth via induction of hydrogen peroxide generation and lipid peroxidation, stimulation of micronutrient absorption as well as reduction of ROS-scavenging enzyme.

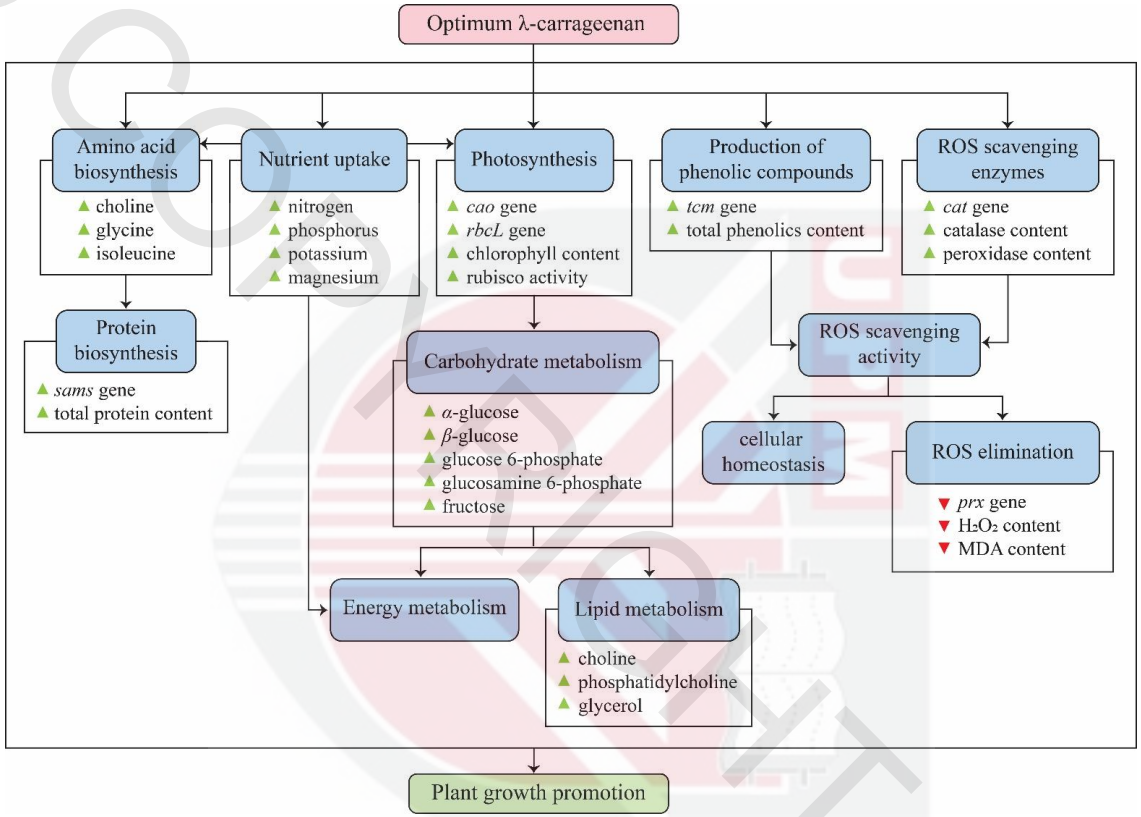


Figure 13: Proposed plant growth-promoting mechanism in banana plants treated with optimum λ-carrageenan. Green triangle represents upregulation while red triangle represents downregulation upon optimum λ-carrageenan treatment.

5.2 Recommendations for future studies

This study established the metabolite profile of banana plants upon treatment with optimum λ-carrageenan via NMR spectroscopy. However, each analytical method has its advantages and limitations. Limitations of NMR spectroscopy (low sensitivity and limited spectral libraries) may hamper the identification of a larger number of secondary metabolites. It is recommended to apply a combination of NMR and mass spectrometry (LC-MS) approaches for better identification of the metabolite profile and underlying mechanism in banana plants treated with λ-carrageenan.

In this study, the potential of λ-carrageenan in enhancing plant growth of *Musa acuminata* cv. Berangan was highlighted. In the future, evaluation of the applicability and effectiveness of λ-carrageenan as a plant growth promoter should be further carried out under field conditions. It is recommended to perform a long-term investigation involving a larger sample size to assess the effects of λ-carrageenan on plant performance in field settings. Furthermore, further evaluations are necessary to determine the feasibility, cost economics, and market acceptance of λ-carrageenan as a growth enhancer in agriculture, in order to expand commercial adoption among farmers.

REFERENCES

Abad, L. V, Aurigue, F. B., Montefalcon, D. R. V, Manguiat, P. H., Carandang, F. F., Mabborang, S. A., Hizon, M. G. S., & Abella, M. E. S. (2018). Effect of radiation-modified kappa-carrageenan as plant growth promoter on peanut (*Arachis hypogaea* L.). *Radiation Physics and Chemistry*, *153*, 239-244.

Abagale, S. A., Woodcock, C. M., Chamberlain, K., Osafo-Acquaah, S., van Emden, H., Birkett, M. A., Pickett, J. A., & Braimah, H. (2019). Attractiveness of host banana leaf materials to the banana weevil, *Cosmopolites sordidus* in Ghana for development of field management strategies. *Pest Management Science*, *75*(2), 549-555.

Abdul Khalil, H. P. S., Tye, Y. Y., Saurabh, C. K., Leh, C. P., Lai, T. K., Chong, E. W. N., Nurul Fazita, M. R., Mohd Hafiidz, J., Banerjee, A., & Syakir, M. I. (2017). Biodegradable polymer films from seaweed polysaccharides: a review on cellulose as a reinforcement material. *Express Polymer Letters*, *11*(4), 244-265.

Abhilash, P. C., Dubey, R. K., Tripathi, V., Gupta, V. K., & Singh, H. B. (2016). Plant growth-promoting microorganisms for environmental sustainability. *Trends in Biotechnology*, *34*(11), 847-850.

Ahmadi, A., Moghadamtousi, S. Z., Abubakar, S., & Zandi, K. (2015). Antiviral potential of algae polysaccharides isolated from marine sources: a review. *BioMed Research International* 2015, 825203.

Ahmed Khan, T., Zakaria, M. E. T., Kim, H., Ghazali, S., & Jamari, S. S. (2019). Carbonaceous microsphere-based superabsorbent polymer as filler for coating of NPK fertilizer: fabrication, properties, swelling, and nitrogen release characteristics. *Journal of Applied Polymer Science*, *137*(8), 48396.

Aina, O., Bakare, O. O., Daniel, A. I., Gokul, A., Beukes, D. R., Fadaka, A. O., Keyster, M., & Klein, A. (2022). Seaweed-derived phenolic compounds in growth promotion and stress alleviation in plants. *Life*, *12*(10), 1548.

Alemu, M. M. (2017). Banana as a cash crop and its food security and socioeconomic contribution : the case of Southern Ethiopia, Arba Minch. *Journal of Environmental Protection*, *8*(3), 319-329.

Almagro, L., Gómez Ros, L. V., Belchi-Navarro, S., Bru, R., Ros Barceló, A., & Pedreño, M. A. (2009). Class III peroxidases in plant defence reactions. *Journal of Experimental Botany*, *60*(2), 377-390.

Ampong, K., Thilakaranthna, M. S., & Gorim, L. Y. (2022). Understanding the role of humic acids on crop performance and soil health. *Frontiers in Agronomy*, *4*, 848621.

Antonio, T. I., Bayla, J. A., Sace, C. F., & Aurique, F. B. (2019). Kale (*Brassica oleracea* var acephala) production using irradiated carrageenan plant growth promoter in passive hydroponics systems. *Philippine Journal of Crop Science*, *44*.

Anwar, A., Yu, X., & Li, Y. (2020). Seed priming as a promising technique to improve growth, chlorophyll, photosynthesis and nutrient contents in cucumber seedlings. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *48*(1), 116-127.

Arancon, N. Q., Edwards, C. A., Lee, S., & Byrne, R. (2006). Effects of humic acids from vermicomposts on plant growth. *European Journal of Soil Biology*, *42*, S65-S69.

Arif, N., Yadav, V., Singh, S., Singh, S., Ahmad, P., Mishra, R. K., Sharma, S., Tripathi, D. K., Dubey, N. K., & Chauhan, D. K. (2016). Influence of high and low levels of plant-beneficial heavy metal ions on plant growth and development. *Frontiers in Environmental Science*, *4*, 69.

Arif, Y., Bajguz, A., & Hayat, S. (2023). *Moringa oleifera* extract as a natural plant biostimulant. *Journal of Plant Growth Regulation*, *42*(3), 1291-1306.

Aurore, G., Parfait, B., & Fahrasmane, L. (2009). Bananas, raw materials for making processed food products. *Trends in Food Science & Technology*, *20*(2), 78-91.

Bahram-Parvar, M., Razavi, S. M. A., Mazaheri Tehrani, M., & Alipour, A. (2013). Optimization of functional properties of three stabilizers and κ-carrageenan in ice cream and study of their synergism. *Journal of Agricultural Science and Technology*, *15*(4), 757-769.

Bassil, E., Coku, A., & Blumwald, E. (2012). Cellular ion homeostasis: emerging roles of intracellular NHX Na+/H+ antiporters in plant growth and development. *Journal of Experimental Botany*, *63*(16), 5727-5740.

Bhuiyan, M. H., Ali, M. S., & Molla, M. M. (2009). Effects of using chemicals and hormones for cultivation and marketing of vegetables and banana. *Final Report (CF 14/07), NFPCSP-FAO, Bangladesh*.

Bi, F., Iqbal, S., Arman, M., Ali, A., & Hassan, M. (2011). Carrageenan as an elicitor of induced secondary metabolites and its effects on various growth characters of chickpea and maize plants. *Journal of Saudi Chemical Society*, *15*(3), 269-273.

Biswal, A. K., Pattanayak, G. K., Pandey, S. S., Leelavathi, S., Reddy, V. S., & Tripathy, B. C. (2012). Light intensity-dependent modulation of chlorophyll *b* biosynthesis and photosynthesis by overexpression of chlorophyllide *a* oxygenase in tobacco. *Plant Physiology*, *159*(1), 433-449.

Blount, J. W., Masoud, S., Sumner, L. W., Huhman, D., & Dixon, R. A. (2002). Over-expression of cinnamate 4-hydroxylase leads to increased accumulation of acetosyringone in elicited tobacco cell-suspension cultures. *Planta*, *214*, 902-910.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Bren d'Amour, C., Reitsma, F., Baiocchi, G., Barthel, S., Güneralp, B., Erb, K., Haberl, H., Creutzig, F., & Seto, K. C. (2016). Future urban land expansion and implications for global croplands. *PNAS*, *114*(34), 8939-8944.

Calderon, R., & Rola, A. C. (2003). *Assessing Benefits and Costs of Commercial Banana Production in The Philippines*. Laguna, Philippines: University of the Philippines Los Baños. Institute of Strategic Planning and Policy Studies.

Castro, J., Vera, J., González, A., & Moenne, A. (2012). Oligo-carrageenans stimulate growth by enhancing photosynthesis, basal metabolism, and cell cycle in tobacco plants (var. Burley). *Journal of Plant Growth Regulation*, *31*, 173-185.

Chakhatrakan, S. (2003). Influences of N fertilizers on the vegetable amaranth production. *Science & Technology Asia*, *8*, 1-5.

Chandini, Kumar, R., Kumar, R., & Prakash, O. (2019). The impact of chemical fertilizers on our environment and ecosystem. In P. Sharma (Ed.), *Research Trends in Environmental Sciences* (pp. 69-86). New Delhi, India: Aiknik Publications.

Chapman, J. M., Muhlemann, J. K., Gayomba, S. R., & Muday, G. K. (2019). RBOH-dependent ROS synthesis and ROS scavenging by plant specialized metabolites to modulate plant development and stress responses. *Chemical Research in Toxicology*, *32*(3), 370-396.

Cheynier, V., Comte, G., Davies, K.M., Lattanzio, V., & Martens, S. (2013). Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry*, *72*, 1-20.

Chiang, C. M., Chen, S. P., Chen, L. F. O., Chiang, M. C., Chien, H. L., & Lin, K. H. (2014). Expression of the broccoli catalase gene (*BoCAT*) enhances heat tolerance in transgenic *Arabidopsis*. *Journal of Plant Biochemistry and Biotechnology*, *23*, 266-277.

Chiappero, J., Cappellari, L. D. R., Sosa, A., Lucas, G., Palermo, T. B., & Banchio, E. (2019). Plant growth promoting rhizobacteria improve the antioxidant status in *Mentha piperita* grown under drought stress leading to an enhancement of plant growth and total phenolic content. *Industrial Crops and Products*, *139*, 111553.

Cho, H. K., Ahn, C. S., Lee, H. S., Kim, J. K., & Pai, H. S. (2013). Pescadillo plays an essential role in plant cell growth and survival by modulating ribosome biogenesis. *The Plant Journal*, *76*(3), 393-405.

Cunha, L., & Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. *Marine Drugs*, *14*(3), 42.

Dallagnol, L. J., Rodrigues, F. A., DaMatta, F. M., Mielli, M. V., & Pereira, S. C. (2011). Deficiency in silicon uptake affects cytological, physiological, and biochemical events in the rice-bipolaris *Oryzae* interaction. *Phytopathology*, *101*(1), 92-104.

De Gara, L., Locato, V., Dipierro, S., & de Pinto, M. C. (2010). Redox homeostasis in plants. The challenge of living with endogenous oxygen production. *Respiratory Physiology & Neurobiology*, *173*, S13-S19.

Dhananjaya, P. S., Ratna, P., Mahesh, S. Y., & Dilip, K. A. (2011). Cyanobacteria-mediated phenylpropanoids and phytohormones in rice (*Oryza sativa*) enhance plant growth and stress tolerance. *Antonie van Leeuwenhoek*, *100*(4), 557-568.

Dordas C. A., & Sioulas, C. (2008). Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. *Industrial Crops and Products*, *27*, 75-85.

Dubois, M., Van den Broeck, L., & Inzé, D. (2018). The pivotal role of ethylene in plant growth. *Trends in Plant Science*, *23*(4), 311-323.

Eco Business Fund (2022). *The World of Bananas*. https://www.ecobusiness.fund/fileadmin/user_upload/Publications/english/Crop_Profiles_LAC_ENG/Crop_Profile_Banana_ENG_June_2022.pdf

Ehlting, J., Hamberger, B., Million-Rousseau, R., & Werck-Reichhart, D. (2006). Cytochromes P450 in phenolic metabolism. *Phytochemistry Reviews*, *5*, 239-270.

Enfissi, E. M., Fraser, P. D., Lois, L. M., Boronat, A., Schuch, W., & Bramley, P. M. (2005). Metabolic engineering of the mevalonate and non-mevalonate isopentenyl diphosphate-forming pathways for the production of health-promoting isoprenoids in tomato. *Plant Biotechnology Journal*, *3*(1), 17-27.

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Trygg, J., Wikstrom, C., & Wold, S. (2006). Multi and mega variate data analysis part 1: Basic principles and applications. California, USA: Umetrics Inc.

Esringü, A., Kaynar, D., Turan, M., & Ercisli, S. (2016). Ameliorative effect of humic acid and plant growth-promoting rhizobacteria (PGPR) on Hungarian vetch plants under salinity stress. *Communications in Soil Science and Plant Analysis*, *47*, 602-618.

Evans, E. A., Ballen, F. H., & Siddiq, M. (2020). Banana production, global trade, consumption trends, postharvest handling, and processing. In M. Siddiq, J. Ahmed, & M. G. Lobo (Eds.), *Handbook of Banana Production, Postharvest Science, Processing Technology, and Nutrition* (pp. 1–18). New Jersey, USA: Wiley.

Evans, J. R. (2013). Improving photosynthesis. *Plant Physiology*, *162*(4), 1780-1793.

Fan, D., Hodges, D. M., Critchley, A. T., & Prithiviraj, B. (2013). A commercial extract of brown macroalga (*Ascophyllum nodosum*) affects yield and the nutritional quality of spinach *in vitro*. *Communications in Soil Science and Plant Analysis*, *44*(12), 1873-1884.

Fang, W. C., Wang, J. W., Lin, C. C., & Kao, C. H. (2001). Iron induction of lipid peroxidation and effects on antioxidative enzyme activities in rice leaves. *Plant Growth Regulation*, *35*, 75-80.

FAO (2017). *Women's employment in the banana industry*. https://www.fao.org/3/i8285e/i8285e.pdf

FAO (2020). *Banana market review snapshot February 2020*. https://www.fao.org/3/ca9212en/ca9212en.pdf

FAO (2022). *The state of world fisheries and aquaculture 2022 (SOFIA)*. https://www.fao.org/3/cc0461en/cc0461en.pdf

Fortunato, A. A., Rodrigues, F. Á., & do Nascimento, K. J. T. (2012). Physiological and biochemical aspects of the resistance of banana plants to Fusarium wilt potentiated by silicon. *Phytopathology*, *102*(10), 957-966.

Gao, J., Wang, H., Yuan, Q., & Feng, Y. (2018). Structure and function of the photosystem supercomplexes. *Frontiers in Plant Science*, *9*, 357.

García, A. C., Berbara, R. L. L., Farías, L. P., Izquierdo, F. G., Hernández, O. L., Campos, R. H., & Castro, R. N. (2012). Humic acids of vermicompost as an ecological pathway to increase resistance of rice seedlings to water stress. *African Journal of Biotechnology*, *11*(13), 3125-3134.

Gobler, C. J. (2020). Climate change and harmful algal blooms: insights and perspective. *Harmful algae*, *91*, 101731.

Goff, S. A. (2011). A unifying theory for general multigenic heterosis: energy efficiency, protein metabolism, and implications for molecular breeding. *New Phytologist*, *189*(4), 923-937.

González, A., Contreras, R. A., & Moenne, A. (2013). Oligo-carrageenans enhance growth and contents of cellulose, essential oils and polyphenolic compounds in *Eucalyptus globulus* trees. *Molecules*, *18*(8), 8740–8751.

González, A., Moenne, F., Gómez, M., Sáez, C. A., Contreras, R. A., & Moenne, A. (2014). Oligo-carrageenan kappa increases NADPH, ascorbate and glutathione syntheses and TRR/TRX activities enhancing photosynthesis, basal metabolism, and growth in *Eucalyptus* trees. *Frontiers in Plant Science*, *5*, 512.

Gou, W., Tian, L., Ruan, Z., Zheng, P., Chen, F., Zhang, L., Cui, Z., Zheng, P., Li, Z., Gao, M., Shi, W., Zhang, L., Liu, J., & Hu, J. (2015). Accumulation of choline and glycinebetaine and drought stress tolerance induced in maize (*Zea mays*) by three plant growth promoting rhizobacteria (PGPR) strains. *Pakistan Journal of Botany*, *47*(2), 581-586.

Guan, Z., Chai, T., Zhang, Y., Xu, J., & Wei, W. (2009). Enhancement of Cd tolerance in transgenic tobacco plants overexpressing a Cd-induced catalase cDNA. *Chemosphere*, *76*(5), 623-630.

Gull, M., & Pasek, M. A. (2021). The role of glycerol and its derivatives in the biochemistry of living organisms, and their prebiotic origin and significance in the evolution of life. *Catalysts*, *11*(1), 86.

Harrison, E. P., Willingham, N. M., Lloyd, J. C., & Raines, C. A. (1997). Reduced sedoheptulose-1, 7-bisphosphatase levels in transgenic tobacco lead to decreased photosynthetic capacity and altered carbohydrate accumulation. *Planta*, *204*, 27-36.

Haydon, M. J., Román, Á., & Arshad, W. (2015). Nutrient homeostasis within the plant circadian network. *Frontiers in Plant Science*, *6*, 299.

Hidalgo, W., Chandran, J. N., Menezes, R. C., Otálvaro, F., & Schneider, B. (2016). Phenylphenalenones protect banana plants from infection by *Mycosphaerella fijiensis* and are deactivated by metabolic conversion. *Plant, cell & environment*, *39*(3), 492-513.

Hilliou, L., Larotonda, F. D., Abreu, P., Abreu, M. H., Sereno, A. M., & Goncalves, M. P. (2012). The impact of seaweed life phase and postharvest storage duration on the chemical and rheological properties of hybrid carrageenans isolated from Portuguese *Mastocarpus stellatus*. *Carbohydrate Polymers*, *87*, 2655-2663.

Huang, H., Ullah, F., Zhou, D. X., Yi, M. & Zhao, Y. (2019). Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in Plant Science*, *10*, 800.

Huang, W., Hu, H., & Zhang, S. B. (2015). Photorespiration plays an important role in the regulation of photosynthetic electron flow under fluctuating light in tobacco plants grown under full sunlight. *Frontiers in Plant Science*, *6*, 621.

Hunt, R. (2012). *Basic Growth Analysis: Plant Growth Analysis for Beginners*. Berlin, Germany: Springer Science & Business Media.

Imeson, A. P. (2000). Carrageenan. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of Hydrocolloids* (pp. 87-102). Cambridge, UK: Woodhead Publishing.

Jaskulak, M., Rorat, A., Grobelak, A., & Kacprzak, M. (2018). Antioxidative enzymes and expression of *rbcL* gene as tools to monitor heavy metal-related stress in plants. *Journal of Environmental Management*, *218*, 71-78.

Jazzara, M., Ghannam, A., Soukkarieh, C., & Murad, H. (2016). Anti-proliferative activity of λ -carrageenan through the induction of apoptosis in human breast cancer cells. *Iranian Journal of Cancer Prevention*, *9*(4), e3836.

Kang, S. M., Khan, A. L., Hamayun, M., Shinwar, Z. K., Kim, Y. H., Joo, G. J., & Lee, I. J. (2012). *Acinetobacter calcoaceticus* ameliorated plant growth and influenced gibberellin and functional biochemical. *Pakistan Journal of Botany*, *44*, 365-372.

Kang, S. M., Radhakrishnan, R., You, Y. H., Joo, G. J., Lee, I. J., Lee, K. E., & Kim, J. H. (2014). Phosphate solubilizing *Bacillus megaterium* mj1212 regulates

endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. *Indian Journal of Microbiology, 54*(4), 427–433.

Kanmani, P., & Rhim, J. (2014). Development and characterization of carrageenan/grapefruit seed extract composite films for active packaging. *International Journal of Biological Macromolecules*, 68, 258-266.

Keena, M., Meehan, M., & Scherer, T. (2022). Environmental implications of excess fertilizer and manure on water quality. North Dakota, USA: North Dakota State University.

Khan, M. I. R., Nazir, F., Asgher, M., Per, T. S., & Khan, N. A. (2015). Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *Journal of Plant Physiology, 173*, 9-18.

Khan, S., Shahnaz, M., Jehan, N., Rehman, S., Shah, M. T., & Din, I. (2013). Drinking water quality and human health risk in Charsadda district, Pakistan. *Journal of Cleaner Production, 60*, 93-101.

Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., Critchley, A. T., Craigie, J. S., Norrie, J., & Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulation, 28*, 386-399.

Kim, H. S., Park, S. J., Hyun, S. H., Yang, S. O., Lee, J., Auh, J. H., Kim, J. H., Cho, S. M., Marriott, P. J., & Choi, H. K. (2011). Biochemical monitoring of black raspberry (*Rubus coreanus* Miquel) fruits according to maturation stage by ^{1}H NMR using multiple solvent systems. *Food Research International, 44*(7), 1977-1987.

Kim, Y. S., Kim, Y. B., Kim, Y., Lee, M. Y., & Park, S. U. (2014). Overexpression of cinnamate 4-hydroxylase and 4-coumaroyl CoA ligase prompted flavone accumulation in *Scutellaria baicalensis* hairy roots. *Natural Product Communications, 9*(6), 1934578X1400900618.

Kitagawa, M., & Jackson, D. (2019). Control of meristem size. *Annual Review of Plant Biology, 70*(1), *269-291*.

Kok, A. D. X., Wan Abdullah, W. M. A. N., Tan, N. P., Ong-Abdullah, J., Sekeli, R., Wee, C. Y., & Lai, K. S. (2020). Growth promoting effects of pluronic f-68 on callus proliferation of recalcitrant rice cultivar. *3Biotech, 10*, 116.

Kok, A. D. X., Wan Abdullah, W. M. A. N., Tang, C. N., Low, L. Y., Yuswan, M. H., Ong-Abdullah, J., Tan, N. P., & Lai, K. S. (2021). Sodium lignosulfonate improves shoot growth of *Oryza sativa* via enhancement of photosynthetic activity and reduced accumulation of reactive oxygen species. *Scientific Reports, 11*, 13226.

Kole, C. (2022). *Genomic Designing for Abiotic Stress Resistant Fruit Crops*. New York, USA: Springer International Publishing.

Koßmann, J., Sonnewald, U., & Willmitzer, L. (1994). Reduction of the chloroplastic fructose-1, 6-bisphosphatase in transgenic potato plants impairs photosynthesis and plant growth. *The Plant Journal*, *6*(5), 637-650.

Kumar, K. P. S., Bhowmik, D., Duraivel, S., & Umadevi, M. (2012). Traditional and medicinal uses of banana. *Journal of Pharmacognosy and Phytochemistry*, *1*(3), 51-63.

Kyakuwaire, M., Olupot, G., Amoding, A., Nkedi-Kizza, P., & Basamba, T. A. (2019). How safe is chicken litter for land application as an organic fertilizer? a review. *International Journal of Environmental Research and Public Health*, 16(19), 3521.

Lavell, A. A., & Benning, C. (2019). Cellular organization and regulation of plant glycerolipid metabolism. *Plant and Cell Physiology*, *60*(6), 1176-1183.

Le Mire, G., Siah, A., Marolleau, B., Gaucher, M., Maumené, C., Brostaux, Y., Massart, S., Brisset, M., & Jijakli, M. H. (2019). Evaluation of λ-carrageenan, CpGODN, *Glycine betaine*, *Spirulina platensis*, and ergosterol as elicitors for control of *Zymoseptoria tritici* in wheat. *Phytopathology*, *109*, 409-417.

Le Moal, M., Gascuel-Odoux, C., Ménesguen, A., Souchon, Y., Étrillard, C., Levain, A., Moatar, F., Pannard, A., Souchu, P., Lefebvre, A., Pinay, G. (2019). Eutrophication: a new wine in an old bottle?. *Science of The Total Environment*, *651*, 1-11.

Less, H., & Galili, G. (2008). Principal transcriptional programs regulating plant amino acid metabolism in response to abiotic stresses. *Plant Physiology*, *147*(1), 316-330.

Li, L. F., Wang, H. Y., Zhang, C., Wang, X. F., Shi, F. X., Chen, W. N., & Ge, X. J. (2013). Origins and domestication of cultivated banana inferred from chloroplast and nuclear genes. *PLoS ONE*, *8*(11), e80502.

Li, L., Ni, R., Shao, Y., & Mao, S. (2014). Carrageenan and its applications in drug delivery. *Carbohydrate Polymers*, *103*, 1-11.

Li, W., Han, Y., Tao, F., & Chong, K. (2011). Knockdown of SAMS genes encoding S-adenosyl-l-methionine synthetases causes methylation alterations of DNAs and histones and leads to late flowering in rice. *Journal of Plant Physiology*, *168*(15), 1837-1843.

Li, Y., Fang, F., Wei, J., Wu, X., Cui, R., Li, G., Zheng, F., & Tan, D. (2019). Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: A three-year experiment. *Scientific reports*, *9*, 12014.

Liang, H. Z., Zhu, F., Wang, R. J., Huang, X. H., & Chu, J. J. (2019). Photosystem II of *Ligustrum lucidum* in response to different levels of manganese exposure. *Scientific Reports*, *9*, 12568.

Lin, L., Yang, H., & Xu, X. (2022). Effects of water pollution on human health and disease heterogeneity: a review. *Frontiers in Environmental Science*, *10*, 880246.

Liu, X. B., Zhang, X. Y., Wang, Y. X., Sui, Y. Y., Zhang, S. L., Herbert, S. J., & Ding, G. (2010). Soil degradation: a problem threatening the sustainable development of agriculture in Northeast China. *Plant, Soil and Environment*, *56*(2), 87-97.

Livak, K. J., & Schmittgen, T. D. (2011). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, *25*(4), 402-408.

López Arnaldos, T., Muñoz, R., Ferrer, M. A., & Calderón, A. A. (2001). Changes in phenol content during strawberry (*Fragaria×ananassa*, cv. Chandler) callus culture. *Physiologia Plantarum*, *113*(3), 315-322.

Lotfi, R., Gharavi-Kouchebagh, P., & Khoshvaghti, H. (2015). Biochemical and physiological responses of *Brassica napus* plants to humic acid under water stress. *Russian Journal of Plant Physiology*, *62*(4), 480-486.

Luo, M., Shao, B., Nie, W., Wei, X., Li, Y., Wang, B., He, Z., Liang, X., Ye, T., & Wei, Y. (2015). Antitumor and adjuvant activity of λ-carrageenan by stimulating immune response in cancer immunotherapy. *Scientific Reports*, *5*, 1-12.

Maathuis, F. J., & Diatloff, E. (2013). Roles and functions of plant mineral nutrients. In F. Maathuis (Ed.), *Plant Mineral Nutrients. Methods in Molecular Biology* (pp. 1-21) New Jersey, USA: Humana Press.

Mani, S. D., & Nagarathnam, R. (2018). Sulfated polysaccharide from *Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva primes defense responses against anthracnose disease of *Capsicum annuum* Linn. *Algal Research*, *32*, 121-130.

Manuhara, G. J., Praseptiangga, D., & Riyanto, R. A. (2016). Extraction and characterization of refined K-carrageenan of red algae [*Kappaphycus Alvarezii* (Doty ex PC Silva, 1996)] originated from Karimun Jawa Islands. *Aquatic Procedia*, *7*, 106-111.

Marchiosi, R., dos Santos, W. D., Constantin, R. P., de Lima, R. B., Soares, A. R., Finger-Teixeira, A., Mota, T. R., de Oliveira, D. M., Foletto-Felipe, M. D. P., Abrahão, J., & Ferrarese-Filho, O. (2020). Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochemistry Reviews*, *19*, 865-906.

Maricle, B. R., White, S. J., Meraz, A., Maforo, N. G., Biggs, T. N., Martin, N. M., & Caudle, K. L. (2014). Effect of ethanol toxicity on enzyme activity in anaerobic respiration in plants. *Transactions of the Kansas Academy of Science*, *117*(3-4), 237–244.

Marikkar, J. M. N., Tan, S. J., Salleh, A., Azrina, A., & Shukri, M. A. M. (2016). Evaluation of banana (*Musa* sp.) flowers of selected varieties for their antioxidative and anti-hyperglycemic potentials. *International Food Research Journal*, *23*(5), 1988-1995.

Mercier, L., Lafitte, C., Borderies, G., Briand, X., Esquerré-Tugayé, M. T., & Fournier, J. (2001). The algal polysaccharide carrageenans can act as an elicitor of plant defence. *New Phytologist*, *149*(1), 43-51.

Messant, M., Hennebelle, T., Guérard, F., Gakière, B., Gall, A., Thomine, S., & Krieger-Liszkay, A. (2022). Manganese excess and deficiency affects photosynthesis and metabolism in *Marchantia polymorpha*. *BioRxiv*, 477552.

Mhamdi, A., & Van Breusegem, F. (2018). Reactive oxygen species in plant development. *Development*, *145*(15), 164376.

Millaleo, R., Reyes-Díaz, M., Ivanov, A. G., Mora, M. L., & Alberdi, M. (2010). Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition*, *10*(4), 470-481.

Mitcham, E. J., & McDonald, R. E. (1993). Respiration rate, internal atmosphere, and ethanol and acetaldehyde accumulation in heat-treated mango fruit. *Postharvest Biology and Technology*, *3*(1), 77-86.

Moenne, A. (2010). Composition and method to stimulate plant growth and defense against pathogens in plants. *U.S. Patent Application 12/666*, 700.

Mohiuddin, A. K. M., Saha, M. K., Hossian, M. S., & Ferdoushi, A. (2014). Usefulness of banana (*Musa paradisiaca*) wastes in manufacturing of bio-products: a review. *The Agriculturists*, *12*(1), 148-158

Moriwaki, T., Yamamoto, Y., Aida, T., Funahashi, T., Shishido, T., Asada, M., Prodhan, S. H., Komamine, A., & Motohashi, T. (2008). Overexpression of the *Escherichia coli* catalase gene, *katE*, enhances tolerance to salinity stress in the transgenic indica rice cultivar, BR5. *Plant Biotechnology Reports*, *2*, 41-46.

Mu, X., Chen, Q., Chen, F., Yuan, L., & Mi, G. (2016). Within-leaf nitrogen allocation in adaptation to low nitrogen supply in maize during grain-filling stage. *Frontiers in Plant Science*, *7*, 699.

Muhardina, V., Ermaya, D., Aisyah, Y., & Haryani, S. (2018). Quality evaluation of probiotic capsule prepared from alginate, carrageenan and tofu waste flour based on bacterial activity and organoleptic test. *International Conference on Agriculture, Environment, and Food Security*, *122*, 012074.

Munsif, R., Zubair, M., Aziz, A., & Zafar, M. N. (2021). Industrial air emission pollution: potential sources and sustainable mitigation. In R. Viskup (Ed.), *Environmental Emissions*. London, UK: IntechOpen.

Naeem, M., Idrees, M., Aftab, T., Alam, M. M., Khan, M., Uddin, M., & Varshney, L. (2015). Radiation processed carrageenan improves plant growth, physiological activities, and alkaloids production in *Catharanthus roseus* L. *Advances in Botany*, *2015*, 150474.

Necas, J., & Bartosikova, L. (2013). Carrageenan: a review. *Veterinarni Medicina*, *58*(4), 187-205.

Nuarsa, I. W., Dibia, I. N., Wikantika, K., Suwardhi, D., & Rai, I. N. (2018). GIS based analysis of agroclimate land suitability for banana plants in Bali province, Indonesia. *HAYATI Journal of Biosciences*, *25*(1), 11-17.

OEC. (n.d.). *Bananas in Malaysia*. https://oec.world/en/profile/bilateral-product/bananas/reporter/mys

Ogunyale, O. G., Fawibe, O. O., Ajiboye, A. A., & Agboola, D. A. (2014). A review of plant growth substances: their forms, structures, synthesis and functions. *Journal of Advanced Laboratory Research in Biology*, *5*(4), 152-168.

Okonya, J. S., Ocimati, W., Nduwayezu, A., Kantungeko, D., Niko, N., Blomme, G., Legg, J. P., & Kroschel, J. (2019). Farmer reported pest and disease impacts on root, tuber, and banana crops and livelihoods in Rwanda and Burundi. *Sustainability*, *11*(6), 1592.

Pacheco, D., Cotas, J., Rocha, C. P., Araújo, G. S., Figueirinha, A., Gonçalves, A. M. M., Bahcevandziev, K., & Pereira, L. (2021). Seaweeds' carbohydrate polymers as plant growth promoters. *Carbohydrate Polymer Technologies and Applications*, *2*, 100097.

Park, N. I., Park, J. H., & Park, S. U. (2011). Overexpression of cinnamate 4-hydroxylase gene enhances biosynthesis of decursinol angelate in *Angelica gigas* hairy roots. *Molecular biotechnology*, *50*, 114-120.

Pedas, P., Husted, S., Skytte, K., & Schjoerring, J. K. (2011). Elevated phosphorus impedes manganese acquisition by barley plants. *Frontiers in Plant Science*, *2*, 37.

Pereira L. (2013). Population studies and carrageenan properties in eight Gigartinales (Rhodophyta) from Western Coast of Portugal. *Scientific World Journal*, 939830.

Pereira, S. F., Gonzalez, R. L., & Dworkin, J. (2015). Protein synthesis during cellular quiescence is inhibited by phosphorylation of a translational elongation factor. *Proceedings of the National Academy of Sciences*, *112*(25), E3274-E3281.

Pettigrew, W. T. (2008). Potassium influences on yield and quality production for maize, wheat, soybean and cotton. *Physiologia Plantarum*, *133*(4), 670-681.

Prasedya, E. S., Miyake, M., Kobayashi, D., & Hazama, A. (2016). Carrageenan delays cell cycle progression in human cancer cells in vitro demonstrated by FUCCI imaging. *BMC Complementary and Alternative Medicine*, *16*(1), 1-9.

Rasheed, F., Markgren, J., Hedenqvist, M., & Johansson, E. (2020). Modeling to understand plant protein structure-function relationships-implications for seed storage proteins. *Molecules*, *25*(4), 873.

Ríos, J. J., Blasco, B., Cervilla, L. M., Rosales, M. A., Sanchez-Rodriguez, E., Romero, L., & Ruiz, J. M. (2009). Production and detoxification of H_2O_2 in lettuce plants exposed to selenium. *Annals of Applied Biology*, *154*(1), 107-116.

Ritchie, H., Roser, M., & Rosado, P. (2022). *Fertilizers*. Our World in Data. https://ourworldindata.org/fertilizers

Rojas-Nery, E., García-Martínez, I., & Totosaus, A. (2015). Effect of emulsified soy oil with different carrageenans in rennet-coagulated milk gels. *International Food Research Journal*, *22*(2), 606-612.

Roje, S. (2006). S-Adenosyl-L-methionine: beyond the universal methyl group donor. *Phytochemistry*, *67*(15), 1686-1698.

Römheld, V., & Kirkby, E. A. (2010). Research on potassium in agriculture: needs and prospects. *Plant and Soil*, *335*, 155-180.

Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., Cheng, J., & Zhang, K. (2019). Jasmonic acid signaling pathway in plants. *International Journal of Molecular Sciences*, *20*(10), 2479.

Sakuraba, Y., Tanaka, R., Yamasato, A., & Tanaka, A. (2009). Determination of a chloroplast degron in the regulatory domain of chlorophyllide *a* oxygenase. *Journal of Biological Chemistry*, *284*, 36689-36699.

Salachna, P., Byczyńska, A., Jeziorska, I., & Udycz, E. (2017). Plant growth of *Verbena bonariensis* L. after chitosan, gellan gum or iota-carrageenan foliar applications. *World Scientific News*, 111-123.

Sangha, J., Kandasamy, S., Khan, W., Bahia, N., Singh, R., Critchley, A., & Prithiviraj, B. (2015). λ-Carrageenan suppresses tomato chlorotic dwarf viroid (TCDVd) replication and symptom expression in tomatoes. *Marine Drugs*, *13*, 2875-2889.

Saucedo, S., Contreras, R. A. & Moenne, A. (2015). Oligo-carrageenan kappa increases C, N and S assimilation, auxin and gibberellin contents, and growth in *Pinus radiata* trees. *Journal of Forestry Research*, *26*, 635-640.

Savci, S. (2012). An agricultural pollutant: chemical fertilizer. *International Journal of Environmental Science and Development*, *3*(1), 77-80.

Searchinger, T., Waite, R., Hanson, C., & Ranganathan, J. (2019). *Creating a sustainable food future*. Washington, USA: World Resources Institutes.

Sergeeva, A. (2017, April 21). *Global banana market to reach 158M tonnes by 2025*. Indexbox. https://www.indexbox.io/blog/global-banana-market-to-reach-158m-tonnes-by-2025/

Shahbandeh, M. (2023). Production of bananas worldwide 2021, by region. https://www.statista.com/statistics/264003/production-of-bananas-worldwide-by-region/

Shi, H., Wu, X., Zhu, Y., Jiang, T., Wang, Z., Li, X., Liu, J., Zhang, Y., Chen, F., Gao, J., Xu, X., Zhang, G., Xiao, N., Feng, X., Zhang, P., Wu, Y., Li, A., Chen, P., & Li, X. (2024). RefMetaPlant: a reference metabolome database for plants across five major phyla. *Nucleic Acids Research*, *52*, D1614-D1628.

Shukla, P. S., Borza, T., Critchley, A. T. & Prithiviraj, B. (2016). Carrageenans from red seaweeds as promoters of growth and elicitors of defense response in plants. *Frontiers in Marine Science*, *3*, 81.

Shukla, P. S., Mantin, E. G., Adil, M., Bajpai, S., Critchley, A. T., & Prithiviraj, B. (2019). *Ascophyllum nodosum*-based biostimulants: sustainable applications in agriculture for the stimulation of plant growth, stress tolerance, and disease management. *Frontiers in Plant Science*, *10*, 655.

Sidhu, J. S., & Zafar, T. A. (2018). Bioactive compounds in banana fruits and their health benefits. *Food Quality and Safety*, *2*(4), 183-188.

Slewinski, T. L., & Braun, D. M. (2010). Current perspectives on the regulation of whole-plant carbohydrate partitioning. *Plant Science*, *178*(4), 341-349.

Soares, F., Fernandes, C., Silva, P., Pereira, L., & Gonçalves, T. (2016). Antifungal activity of carrageenan extracts from the red alga *Chondracanthus teedei* var. *lusitanicus*. *Journal of Applied Phycology*, *28*, 2991–2998.

Soares, T. F. S. N., da Silva, A. V. C., & Muniz, E. N. (2021). *Moringa* leaf extract: a cost-effective and sustainable product to improve plant growth. *South African Journal of Botany*, *141*, 171-176.

Solomon Wisdom, G. O., Ndana, R. W., & Abdulrahim, Y. (2012). The comparative study of the effect of organic manure cow dung and inorganic fertilizer NPK on the growth rate of maize (*Zea mays* L). *International Research Journal of Agricultural Science and Soil Science*, *2*(12), 516-519.

Stadnik, M. J., & Freitas, M. B. (2014). Algal polysaccharides as source of plant resistance inducers. *Tropical Plant Pathology*, *39*(2), 111-118.

Stover, R. H., & Simmonds, N. W. (1987). *Bananas* (3rd). Harlow, UK: Longman Scientific and Technical.

Tan, B. C. (2022). Can banana be a success story for Malaysia?. *Journal of Agribusiness Marketing*, *9*, 13-22.

Tanaka, R., Koshino, Y., Sawa, S., Ishiguro, S., Okada, K., & Tanaka, A. (2001). Overexpression of chlorophyllide a oxygenase (CAO) enlarges the antenna size of photosystem II in *Arabidopsis thaliana*. *The Plant Journal*, *26*, 365-373.

Thirumurugan, D., Cholarajan, A., Raja, S. S., & Vijayakumar, R. (2018). An introductory chapter: secondary metabolites. In R. Vijayakumar & S. S. Raja (Eds.), *Secondary Metabolites- Sources and Applications* (pp. 1-21). London, UK: IntechOpen.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. In J. Schrader & J. Bohlmann (Eds.), *Biotechnology of Isoprenoids* (pp. 63-106). New York, USA: Springer.

Tian, X., Wang, G., Zhu, R., Yang, P., Duan, L., & Li, Z. (2008). Conditions and indicators for screening cotton (*Gossypium hirsutum* L.) varieties tolerant to low potassium. *Acta Agronomica Sinica*, *34*, 1435-1443.

Tinzaara, W., Ocimati, W., Kikulwe, E., & Otieno, G. (2018). Challenges and opportunities for smallholders in banana value chains. In G. Kema & A. Drenth (Eds.), *Achieving Sustainable Cultivation of Bananas* (pp. 65-90). Cambridge, UK: Burleigh Dodds Science Publishing.

Treutter, D. (2010). Managing phenol contents in crop plants by phytochemical farming and breeding-visions and constraints. *International Journal of Molecular Sciences*, *11*(3), 807-857.

United Nations. (2013, December 3). *World must sustainably produce 70 per cent more food by mid-century-UN report.* https://news.un.org/en/story/2013/12/456912

Varma, V., & Bebber, D. P. (2019). Climate change impacts on banana yields around the world. *Nature Climate Change*, *9*(10), 752-757.

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science*, *151*(1), 59-66.

Vera, J., Castro, J., Contreras, R. A., González, A., & Moenne, A. (2012). Oligo-carrageenans induce a long-term and broad-range protection against pathogens in tobacco plants (var. Xanthi). *Physiological and Molecular Plant Pathology*, *79*, 31-39.

Viljoen, A., Mahuku, G., Massawe, C., Ssali, R. T., Kimunye, J., Mostert, G., Ndayihanzamaso, P., & Coyne, D. L. (2017). *Banana Diseases and Pests: Field Guide for Diagnostics and Data Collection*. Ibadan, Nigeria: International Institute of Tropical Agriculture.

Voora, V., Bermúdez, S., Farrell, J. J., Larrea, C., & Luna, E. (2023). *Global market report: Banana prices and sustainability*. International Institute for Sustainable Development. https://www.iisd.org/system/files/2023-03/2023-global-market-report-banana.pdf

Wan Abdullah, W. M. A. N., Saidi, N. B., Yusof, M. T., Wee, C. Y., Loh, H. S., Ong-Abdullah, J., & Lai, K. S. (2022). Vacuolar processing enzymes modulating susceptibility response to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 infections in banana. *Frontiers in Plant Science*, *12*, 769855.

Wang, J., Song, L., Gong, X., Xu, J., & Li, M. (2020). Functions of jasmonic acid in plant regulation and response to abiotic stress. *International Journal of Molecular Sciences*, *21*(4), 1446.

Wang, W. B., Kim, Y. H., Lee, H. S., Kim, K. Y., Deng, X. P., & Kwak, S. S. (2009). Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant Physiology and Biochemistry*, *47*(7), 570-577.

Wang, X. (2004). Lipid signaling. *Current Opinion in Plant Biology*, *7*(3), 329-336.

Wei, H., Xu, C., Movahedi, A., Sun, W., Li, D., & Zhuge, Q. (2019). Characterization and function of 3-hydroxy-3-methylglutaryl-coA reductase in *Populus trichocarpa*: overexpression of *PtHMGR* enhances terpenoids in transgenic poplar. *Frontiers in Plant Science*, *10*, 1476.

Wei, X., Deng, X., Xiang, W., Lei, P., Ouyang, S., Wen, H., & Chen, L. (2018). Calcium content and high calcium adaptation of plants in karst areas of southwestern Hunan, China. *Biogeosciences*, *15*(9), 2991-3002.

Wishart, D. S., Guo, A., Oler, E., Wang, F., Anjum, A., Peters, H., Dizon, R., Sayeeda, Z., Tian, S., Lee, B. L., Berjanskii, M., Mah, R., Yamamoto, M., Jovel, J., Torres-Calzada, C., Hiebert-Giesbrecht, M., Lui, V. W., Varshavi, D., Varshavi, D., Allen, D., Arndt, D., Khetarpal, N., Sivakumaran, A., Harford, K., Sanford, S., Yee, K., Cao, X., Budinski, Z., Liigand, J., Zhang, L., Zheng, J., Mandal, R., Karu, N., Dambrova, M., Schiöth, H. B., Greiner, R., & Gautam, V. (2022). HMDB 5.0: the human metabolome database for 2022. *Nucleic Acids Research*, *50*, D622-D631.

Wullschleger, S., Loewith, R., & Hall, M. N. (2006). TOR signaling in growth and metabolism. *Cell*, *124*(3), 471-484.

Xu, F., Shi, L., Chen, W., Cao, S., Su, X., & Yang, Z. (2014). Effect of blue light treatment on fruit quality, antioxidant enzymes and radical-scavenging activity in strawberry fruit. *Scientia Horticulturae*, *175*, 181-186.

Xu, J. W., Xu, Y. N., & Zhong, J. J. (2012). Enhancement of ganoderic acid accumulation by overexpression of an n-terminally truncated 3-hydroxy-3-methylglutaryl coenzyme A reductase gene in the basidiomycete *Ganoderma lucidum*. *Applied and Environmental Microbiology*, *78*(22), 7968-7976.

Yasin, H., Babji, A. S., & Ismail, H. (2016). Optimization and rheological properties of chicken ball as affected by κ-carrageenan, fish gelatin and chicken meat. *LWT - Food Science and Technology*, *66*, 79-85.

Yildiztekin, M., Tuna, A. L., & Kaya, C. (2018). Physiological effects of the brown seaweed (*Ascophyllum nodosum*) and humic substances on plant growth, enzyme activities of certain pepper plants grown under salt stress. *Acta Biologica Hungarica*, *69*, 325-335.

You, J., & Chan, Z. (2015). ROS regulation during abiotic stress responses in crop plants. *Frontiers in Plant Science*, *6*, 1092.

Yu, J. G., Lee, G. H., & Park, Y. D. (2012). Physiological role of endogenous S-adenosyl-L-methionine synthetase in Chinese cabbage. *Horticulture, Environment, and Biotechnology*, *53*, 247–255.

Zhang, C., Hu, R., Shi, G., Jin, Y., Robson, M. G., & Huang, X. (2015). Overuse or underuse? An observation of pesticide use in China. *Science of The Total Environment*, *538*, 1-6.

Zhang, S. (2011). *Air quality and community health impact of animal manure management*. Vancouver, Canada: National Collaborating Centre for Environmental Health.

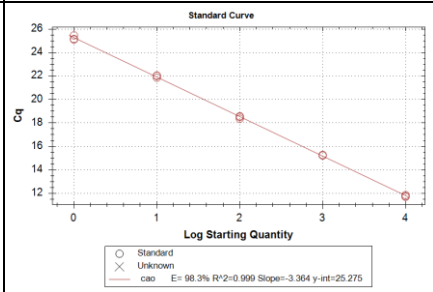
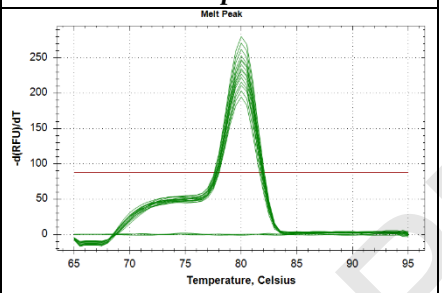
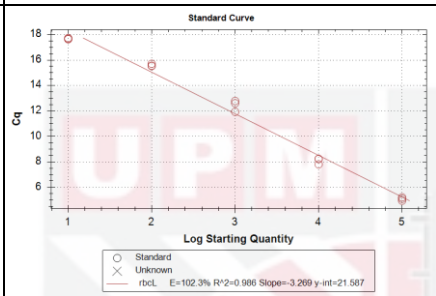
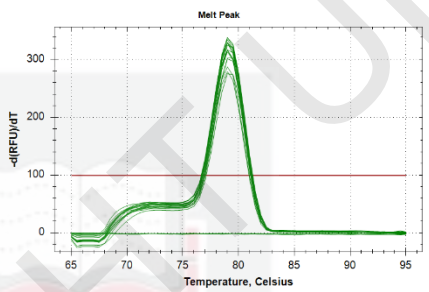
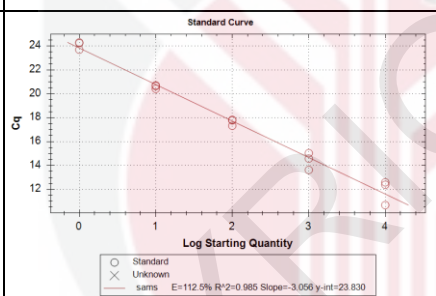
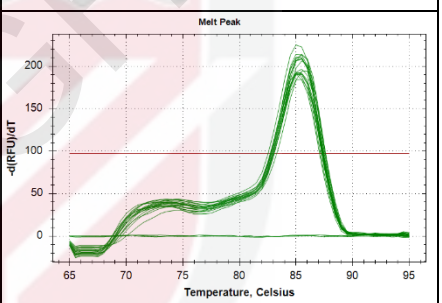
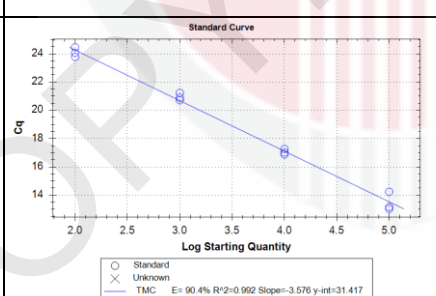
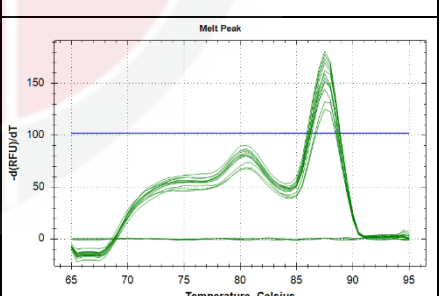
Zhong, M., Yuan, Y., Shu, S., Sun, J., Guo, S., Yuan, R., & Tang, Y. (2016). Effects of exogenous putrescine on glycolysis and Krebs cycle metabolism in cucumber leaves subjected to salt stress. *Plant Growth Regulation*, *79*, 319-330.

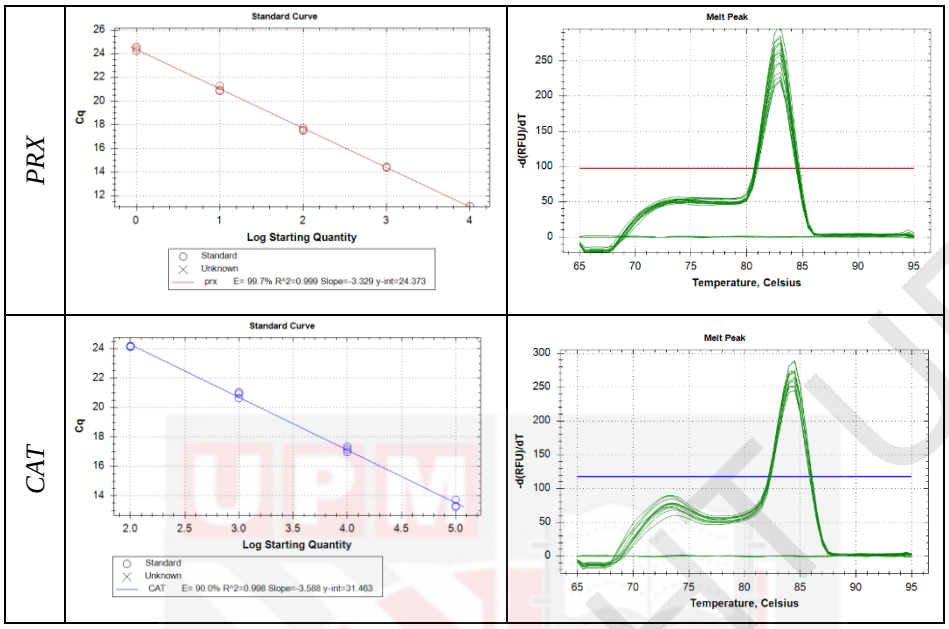
Zi, X., Zhou, S., & Wu, B. (2022). Alpha-linolenic acid mediates diverse drought responses in maize (*Zea mays* L.) at seedling and flowering stages. *Molecules*, *27*(3), 771.

APPENDICES

Appendix 1.0: List of primers used for RT-qPCR analysis.

Primer name	Sequence (5'-3')	Annealing temperature (°C)
Target gene		
CAO	Fw: GCAAGAAGGAATGGTTTGGA	60.0
	Rv: TATCCAAAAGAAGCCCATGC	
RBCL	Fw: ACAAAGGGCGATGCTACCAC	60.0
	Rv: TGGGAATTCGCAGATCCTCC	
SAMS	Fw: GGACTGCTGCTCGATGTTGA	60.0
	Rv: ATCACCACCAAGGCCAATGT	
TCM	Fw: TGTCGAAGCTGTGTGGCAAG	60.0
	Rv: GGAAGATTTCGCCGAAGCG	
PRX	Fw: CAGACGAGATAGCAAGGAAAGCCAC	60.0
	Rv: TCCCACGAGGTTGACACAG	
CAT	Fw: GGCGTCAACACCTACACCTT	60.0
	Rv: AGCTTCCACTCCGGGTAGTT	
Housekeeping gene		
GAPDH	Fw: CCAGCAAGGATGCCCCAATGT	60.0
	Rv: CTGCACAACCAACTGTCTTGCT	
UBQ	Fw: TCCAGCGGCTCCAAGTTCTC	60.0
	Rv: GCGAGCGTTTGGTCGTCATTC	

	Standard curve	*Melt peak*
CAO		
RBCL		
SAMS		
TCM		



Appendix 2.0: Validation of primers used in RT-qPCR analysis through standard curve and melt peak analysis.

Appendix 3.0: Operating parameters of ICP-OES.

Radiofrequency power	1100 W
Plasma gas flow rate	15.0 L min^{-1}
Auxiliary gas flow rate	1.50 L min^{-1}
Replicate read time	3 s
Delay	15 s
Sample uptake delay	30 s
Pump rate	15 rpm
Rinse time	20 s
Replicates	3

BIODATA OF STUDENT

Thye Kah Lok was born in Ipoh, Perak on 21st March 1994. She started her primary education in S.J.K. (C) Perak from 2001-2006 and continued her secondary education in S.M.J.K. Perempuan Perak from 2007-2011. Then, she completed her sixth form in S.M.K. Raja Perempuan from 2012-2013. After that, she pursued her study in Bachelor Science (Honours) Cell and Molecular Biology at Universiti Putra Malaysia. She graduated with First Class Honours in 2018. In the same year, she began her Master's degree at the same university. During her Master's study, she has been focusing her research interest in the field of plant biotechnology and molecular biology.

LIST OF PUBLICATIONS

Research paper

Thye, K. L., Wan Abdullah, W. M. A. N., Balia Yusof, Z. N., Wee, C. Y., Ong-Abdullah, J., Loh, J. Y., Cheng, W. H., Lamasudin, D. U., & Lai, K. S. (2022). λ-Carrageenan promotes plant growth in banana via enhancement of cellular metabolism, nutrient uptake, and cellular homeostasis. *Scientific Reports*, *12*, 19639.

Thye, K. L., Wan Abdullah, W. M. A. N., Ong-Abdullah, J., Lamasudin, D. U., Wee, C. Y., Mohd Yusoff, M. H. Y., Loh, J. Y., Cheng, W. H., & Lai, K. S. (2023). Calcium lignosulfonate modulates physiological and biochemical responses to enhance shoot multiplication in *Vanilla planifolia* Andrews. *Physiology and Molecular Biology of Plants*, *29*(3), 377-392.

Conferences

Thye, K. L., Wan Abdullah, W. M. A. N., Ong-Abdullah, J., Lamasudin, D. U., Balia Yusof, Z. N., Wee, C. Y., & Lai, K. S. (2020). Effect of lambda-carrageenan on growth of *Musa acuminata* cv. Berangan. *Asian Regional Conference on Systems Biology 2020*, Ombak Villa, Langkawi, Malaysia. 2-4 March 2020. (poster presentation)

Thye, K. L., Wan Abdullah, W. M. A. N., Ong-Abdullah, J., Lamasudin, D. U., Balia Yusof, Z. N., Wee, C. Y., & Lai, K.S. (2021). Lambda-carrageenan promotes growth and stress tolerance of banana through enhancement of carbon fixation, cell metabolism and ROS scavenging activity. *The 7th International Biotechnology Symposium*, Universiti Malaysia Sabah. 19-21 August 2021. (poster presentation; Best Poster Presenter Award)

Thye, K. L., Wan Abdullah, W. M. A. N., Balia Yusof, Z. N., Wee, C. Y., Ong-Abdullah, J., Lamasudin, D. U., & Lai, K. S. (2022). Assessing the effects of λ-carrageenan to enhance plant growth on *Musa acuminata* cv. Berangan. *The 4th International Symposium on Functional Genomics and Structural Biology*, Universiti Putra Malaysia. 28 November-2 December 2022. (poster presentation)

Thye, K. L., Wan Abdullah, W. M. A. N., Ong-Abdullah, J., Balia Yusof, Z. N., Wee, C. Y., Lamasudin, D. U., & Lai, K. S. (2023). Plant growth promoting effects of lambda-carrageenan on *Musa acuminata* via upregulation of cellular metabolism, nutrients uptake, and maintenance of cellular homeostasis. *International Postgraduate Symposium in Biology, Biotechnology and Bioengineering 2023*, Universiti Kebangsaan Malaysia. 10-11 January 2023. (oral presentation)