APPLICATION OF LIGHT-EMITTING DIODE WITH PLANT GROWTH PROMOTING RHIZOBACTERIA AND ARBUSCULAR MYCORRHIZA FUNGI FOR ECONOMIC CROP SEEDLING PRODUCTION



A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology Suranaree University of Technology Academic Year 2021 การประยุกต์ใช้แสงแอลอีดีร่วมกับแบคทีเรียที่ส่งเสริมการเจริญเติบโตของพืช และเชื้อราอาร์บัสคูลาร์ไมคอร์ไรซา เพื่อการผลิตต้นกล้าพืชเศรษฐกิจ



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาเทคโนโลยีชีวภาพ มหาวิทยาลัยเทคโนโลยีสุรนารี ปีการศึกษา 2564

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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อภิสิทธิ์ ทรงแสง : การประยุกต์ใช้แสงแอลอีดีร่วมกับแบคทีเรียที่ส่งเสริมการเจริญเติบโตของพืช และเชื้อราอาร์บัสคูลาร์ไมคอร์ไรซา เพื่อการผลิตต้นกล้าพืชเศรษฐกิจ (APPLICATION OF LIGHT-EMITTING DIODE WITH PLANT GROWTH PROMOTING RHIZOBACTERIA AND ARBUSCULAR MYCORRHIZA FUNGI FOR ECONOMIC CROP SEEDLING PRODUCTION) อาจารย์ที่ปรึกษา : ศาสตราจารย์ ดร. หนึ่ง เตียอำรุง, 173 หน้า

คำสำคัญ: แสงแอลอีดี/แบคทีเรียที่ส่งเสริมการเจริญเติบโตของพืช/เชื้อราอาร์บัสคูลาร์ไมคอร์ไรซา/ ต้นกล้าพืช

้ ปัจจุบันมีการนำเทคโนโลยีหลายอย่างม<mark>าป</mark>ระยุกต์ใช้เพื่อส่งเสริมการพัฒนา และการเจริญเติบ โต ้ของพืช เช่น แสงแอลอีดี และจุลินทรี<mark>ย์ที่มีประ</mark>โยชน์ต่อพืช การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อ พัฒนาเทคโนโลยีที่เหมาะสม โดยการใช้<mark>ป</mark>ระโยช<mark>น์</mark>จากแสงแอลอีดีร่วมกับเชื้อแบคทีเรียที่ส่งเสริมการ เจริญเติบโตของพืช (PGPR) และเช<mark>ื้อร</mark>าอาบัสคู<mark>ลาร์</mark>ไมคอร์ไรซา (AMF) เพื่อการผลิตต้นกล้าพืช ้ เศรษฐกิจให้มีคุณภาพ ผลการทดลอ<mark>งแส</mark>ดงให้เห็นว<mark>่า แ</mark>สงแอลอีดีสีแดง (R) และสีน้ำเงิน (B), เชื้อ PGPR และ เชื้อ AMF สามารถส่<mark>งเส</mark>ริมการเจริญเติบโต<mark>ของ</mark>ต้นกล้าได้ โดยแสงแอลอีดีที่ความเข้มแสง 200 µmol/m²/s เหมาะที่สุ<mark>ดในก</mark>ารส่งเสริมเจริญเติบโตของต้นกล้ามะเขือเทศ และพริก ในขณะที่ แสงแอลอีดีที่ความเข้มแสง 300 µmol/m²/s เหมาะที่สุด ในการเจริญเติบโตของต้นกล้าเมล่อน และ ้ผักกาดเขียวปลี และคว<mark>ามเ</mark>ข้ม<mark>แสงที่ 400 µmol/m²/s</mark> เหม<mark>าะที่</mark>สุดในการเจริญเติบโตของต้นกล้า คะน้าฮ่องกง จากนั้นค<mark>วามเข้</mark>มแสงแอลอีดีที่เหมาะสมได้ถูกนำไปทด</mark>สอบอัตราส่วนของแสงที่เหมาะสม ้กับพืชต่าง ๆ พบว่าแสงแ<mark>อลอีดีที่อัตราส่วน R60:B40 เป็นอัตรา</mark>ส่วนเหมาะสมที่สุดต่อการเจริญเติบโต ของต้นกล้ามะเขือเทศ เมล่อน และคะน้ำฮ่องกง ในขณะที่แสงแอลอีดีที่อัตราส่วน R50:B50 เป็น ้อัตราส่วนเหมาะสมที่สุดต่อการเจริญเติบโตของต้นกล้าพริก และผักกาดเขียวปลี หลังจากนั้นใช้อัตรา ส่วนแสงที่เหมาะสมของพืชแต่ละชนิดไปทดสอบหาระยะเวลาการให้แสงที่เหมาะสมต่อต้นกล้า ผลการ ทดลองพบว่า การให้แสงแอลอีดีที่ 20 ชั่วโมงต่อวัน เหมาะสำหรับการเจริญเติบโตของต้นกล้ามะเขือเทศ และเมล่อนที่สุด ในขณะที่การให้แสงแอลอีดีที่ 18 ชั่วโมงต่อวัน เหมาะสำหรับการเจริญเติบโตของ ต้นกล้าพริก และผักกาดเขียวปลีที่สุด และการให้แสงแอลอีดีที่ 12 ชั่วโมงต่อวัน เหมาะสำหรับการ เจริญเติบโตของต้นกล้าคะน้าฮ่องกง หลังจากนั้นทำการทดสอบประสิทธิภาพของเชื้อ PGPR ทั้งหมด 8 สายพันธุ์ ต่อการส่งเสริมการเจริญเติบโตของต้นกล้า และคัดเลือก 2 สายพันธุ์ไปปลูกเชื้อร่วมกับต้นกล้า ภายใต้แสงแอลอีดีที่เหมาะสมต่อการเจริญเติบโต ซึ่งการปลูกเชื้อ PGPR ร่วมกับต้นกล้าที่ปลูกภายใต้ ้แสงแอลอีดีพบว่า ต้นกล้ามะเขือเทศมีดัชนีความแข็งแรงสูงที่สุดเมื่อปลูกเชื้อ Bacillus velezensis SD10 ร่วม ในขณะที่ต้นกล้าเมล่อน และพริกมีดัชนีความแข็งแรงสูงที่สุดเมื่อปลูกเชื้อ Bradyrhizobium sp. SUTN9-2ร่วม และต้นกล้าคะน้ำฮ่องกงมีดัชนีความแข็งแรงสูงที่สุดเมื่อปลูกด้วยเชื้อ Bacillus velezensis S141 ร่วม อย่างไรก็ตาม ต้นกล้าผักกาดเขียวปลีมีดัชนีความแข็งแรงสูงที่สุดเมื่อไม่ปลูก ร่วมกับเชื้อ ในส่วนของการปลูกด้วยเชื้อ *Rhizophagus irregularis* (AMF) ร่วมกับต้นกล้าที่ผลิต ภายใต้แสงที่แตกต่างกันต่อการเจริญเติบโตของต้นกล้าหลังจากย้ายปลูกในสภาวะโรงเรือน 30 วัน พบว่าต้นกล้ามะเขือเทศที่ปลูกเชื้อ SD10 ภายใต้แสงแอลอีดี และปลูกเชื้อ AMF ร่วม ส่งผลให้ต้น มะเขือเทศ มีมวลชีวภาพสูงที่สุด อย่างไรก็ตามการเข้าอยู่อาศัยของเชื้อ AMF ลดลงอย่างมีนัยสำคัญ ในขณะที่ต้นเมล่อนมีมวลชีวภาพสูงที่สุด อย่างไรก็ตามการเข้าอยู่อาศัยของเชื้อ AMF ลดลงอย่างมีนัยสำคัญ ในขณะที่ต้นเมล่อนมีมวลชีวภาพสูงที่สุด อย่างไรก็ตามการเข้าอยู่อาศัยของเชื้อ AMF ลดลงอย่างมีนัยสำคัญ ในขณะที่ต้นเมล่อนมีมวลชีวภาพสูงที่สุดเมื่อปลูกเชื้อ SUTN9-2 ร่วมกับต้นกล้าภายใต้แสงแอลอีดี และ ต้นพริกมีมวลชีวภาพสูงที่สุดเมื่อปลูกเชื้อ SUTN9-2 ร่วมกับต้นกล้าภายใต้แสงแอลอีดี และ ปุทดสอบการให้ผลผลิตในแปลงทดลอง ซึ่งผลการทดลองแสดงให้เห็นว่ากล้ามะเชือเทศ และคะน้าฮ่องกง ที่ผลิตภายใต้สภาวะแสงที่เหมาะสมสามารถส่งเสริมให้ผลผลิตเพิ่มขึ้นประมาณ 16% และ 13.82% ตามลำดับ นอกจากนี้ ต้นกล้ามะเขือเทศที่ปลูกภายใต้แสงแอลอีดีที่เหมาะสม ส่งผลให้ยีนที่เกี่ยวข้อง กับกระบวนการสังเคราะห์ด้วยแสงประกอบด้วย *psbA*, *psbB*, *fdx*, *atpB*, และ *rbc*L มีการ แสดงออกเพิ่มขึ้นอย่างมีนัยสำคัญ

ดังนั้นผลการทดลองเหล่านี้ ให้ข้อมูลที่สำคัญสำหรับสภาวะแสงที่เหมาะสมที่สุด ในระบบ การผลิตกล้าไม้คุณภาพสูง และการปลูกเชื้อ PGPR ร่วมกับต้นกล้า และร่วมกับเชื้อ AMF สามารถ ช่วยเพิ่มการเจริญเติบโตของพืชได้ทั้งระยะต้นกล้า และหลังย้ายปลูก ต้นกล้าคุณภาพสูงที่ผลิตโดยใช้ แสง LED และปลูกเชื้อ PGPR หรือร่วมกับ AMF พบว่ามีศักยภาพในการเพิ่มผลผลิต



สาขาวิชาเทคโนโลยีชีวภาพ ปีการศึกษา 2564

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APISIT SONGSAENG : APPLICATION OF LIGHT-EMITTING DIODE WITH PLANT GROWTH PROMOTING RHIZOBACTERIA AND ARBUSCULAR MYCORRHIZA FUNGI FOR ECONOMIC CROP SEEDLING PRODUCTION. THESIS ADVISOR : PROF. NEUNG TEAUMROONG, Ph.D., 173 PP.

Keyword: Light-emitting diode/plant growth promoting Rhizobacteria/Arbuscular Mycorrhiza Fungi/plant seedling

Currently, many technologies have been applied to enhance plant growth and development such as Light-emitting diode (LED) and beneficial plant microorganism. The aim of this study is to develop appropriate technology by incorporating the benefits of LED light, Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhiza Fungi (AMF) on the quality of economic crop seedling production. The results demonstrated that the red (R) and blue (B) LED light, PGPR, and AMF showed the effects on plant seedling growth. The intensity of LED light at 200 µmol/m²/s was the most appropriate for tomato and chili seedlings. While, LED light at 300 µmol/m²/s was the best light intensity for melon and mustard green seedlings, and at 400 µmol/m²/s was best light intensity for Chinese kale seedling. Then the optimum light intensity was used to determine the optimum light ratio according to suitable light intensity of each plant variety. The results showed that the LED light ratio at R60:B40 was the best light for tomato, melon and Chinese kale seedlings. While the LED light ratio at R50:B50 was the most appropriate for chili and mustard green seedlings. After that, the optimum light ratio was used to determine the optimum light photoperiod according to the suitable light ratio of each plant variety. The LED light photoperiod at 20 h/D was the most appropriate for tomato and melon seedlings, 18 h/D was the most appropriate for chili and mustard green seedlings and 12 h/D was the best for Chinese kale seedling. The investigation of capability 8 PGPR strains to promote plant seedling was conducted and 2 strains were selected to inoculate with seedlings under each optimized LED condition. The results showed that the highest health index tomato seedling was found when Bacillus velezensis SD10 was inoculated, while the highest health index of melon and chili seedlings was obvious from Bradyrhizobium sp. SUTN9-2 inoculation. The

highest health index of Chinese kale seedling was found when *Bacillus velezensis* S141 was inoculated. However, the highest health index of mustard green was found under LED illuminated without inoculation. The *Rhizophagus irregularis* (AMF) inoculation with seedlings was produced under different conditions on seedling growth after transplant to greenhouse for 30 days. The LED illuminated tomato seedling inoculated with SD10 in combination with AMF showed the highest biomass. However, it significantly reduced root colonization. While the highest biomass of melon was found in LED illuminated chili seedling inoculated with SUTN9-2. Finally, the LED illuminated chili seedling inoculated with SUTN9-2 in combination with AMF showed the highest biomass accumulation. After that tomato and Chinese kale seedlings were produced under field conditions. This experiment demonstrated that the tomato and Chinese kale produced under optimum conditions. In addition, the optimum LED illuminated tomato seedling resulted in photosynthesis related genes including *psbA*, *psbB*, *fdx*, *atpB*, and *rbcL* genes were significantly up-regulated.

Therefore, these results provided information for optimum lighting conditions for a high quality seedling production system. In addition, the inoculation of PGPR with a seedling and a combination of AMF could enhance the growth of plants in the seedling and post-transplanting state. Moreover, the high quality seedling that was produced using LED light and inoculated with PGPR or in combination with AMF showed the potential for increasing crop yield production.

้^{วักยา}ลัยเทคโนโลยีสุรั

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LIST OF ABBREVIATIONS

LED	=	light-emitting diode
R	=	red light
В	=	blue light
PGPR	=	Plant Growth Promoting Rhizobacteria
AMF	=	Arbuscu <mark>lar</mark> Mycorrhizal Fungi
ILs	=	Includ <mark>e inca</mark> ndescent Lamps
FLs	=	Fluorescent Lamp
HPMLs	=	High-Pressure Mercury Lamps
HPSLs	=	High-Pressure Sodium Lamps
MHLs	=	Metal-Halide Lamps
PAR	=	Photosynthetically Active Radiation
$C_6H_{12}O_6$	=	glucose
H_2O_2	=	water
CO ₂	- 21	carbon dioxide
O ₂	=	oxygen
PSI	=	photosystem I
PSII		photosystem II
NADPH	= 15he	Nicotinamide adenine dinucleotide phosphate
ATP	=	Adenosine triphosphate
Fd	=	ferredoxin
PQ	=	plastoquinone
PC	=	plastocyanin
Cyt b ₆ f	=	cytochrome b6f complex
FNR	=	ferredoxin-NADP reductase
Cyt c ₆	=	Cytochrome c6
PFLP	=	Plant ferredoxin-like protein
RuBP	=	Ribulose-1,5-bisphosphate
RuBisCo	=	Ribulose-1,5-bisphosphate carboxylase oxygenase

LIST OF ABBREVIATIONS (Continued)

G ₃ P	=	Glyceraldehyde 3-phosphate
PGAL	=	Phosphoglyceraldehyde
PGA	=	Phosphoglycerate
SOD	=	Superoxide dismutase
CAT	=	Catalase
POD	=	Peroxidase
RbcL	=	Ribulos <mark>e b</mark> isphosphate carboxylase large subunit gene
RbcS	=	Ribulo <mark>se bisp</mark> hosphate carboxylase small subunit gene
fdx	=	Ferredoxin gene
atpB	=	ATP synthase subunit beta subunit genes
PabA	=	G <mark>ene</mark> encode <mark>d D</mark> 1 protein
PsbB	=	Gene encoded CP47 protein
ACT	=	Actin gene
SOD	=	Superoxide dismutase
APX	- 2	Ascorbate peroxidase
PCR	=	Polymerase Chain Reaction
qRT-PCR	=	Real-Time Quantitative Reverse Transcription PCR
OD	E V	Optical Density
LB	=77	Luria-Bertani medium
YM	= "One	Yeast extract-mannitol medium
NaOCl	=	Sodium hypochlorite
°C	=	Degree Celsius
%	=	Percent
g	=	gram
mg	=	milligram
μg	=	microgram
μι	=	microliter
μm	=	micrometer
kg	=	kilogram

LIST OF ABBREVIATIONS (Continued)

cm	=	centimeters
µmole/m ² /s	=	micromole per square meter per second
h/D	=	hour/Day
ha	=	hectare
SD	=	standard Deviation



CHAPTER I

INTRODUCTION

1.1 Significant of study

Agriculture has been considered as an important source of food production in Thailand because Thailand has various suitable resources for agriculture. However, Thailand has imported seeds of several economic crops for agriculture. In 2018, there was a reported area of 107,484 rai of large chili peppers (Capsicum sp.) planted with a total yield of 173,304 tons. The average selling price of large chili peppers was 26.10 Baht/kg. Moreover, tomatoes (Solanum lycopersicum L.) have wide varieties, such as for fresh consumption and for feeding into factories for processing. In 2019, the areas for growing tomatoes for fresh consumption and feeding into processing factories are 9,638 and 12,177 rai, respectively, with the harvested of fresh consumption are 14,767 and 20,206 tons delivered to the factory, respectively. The selling price of fresh fruit and factory tomatoes is 13.44 and 3.31 Baht/kg, respectively (Department of Agricultural Extension, 2020). Those plant cultivars are group of the economic crops of Thailand. Problems with seeds germination and seedling vigor are the most common in plants that kept their seeds for a long time. The seeds were also planted and germinated under unsuitable conditions. Most of the imported seeds are expensive, especially the purebred parents, which are often limitations on germination and weak.

The cultivation of many economically valued crops requires good seedlings to ensure that the plant can continue to grow when planted and obtain the product efficiently. The present approach for seedling production found that elongation, growth-retarding of seedlings when light is insufficient, particularly during rainy seasons (Chen et al., 2014; Ren et al., 2018; Wei et al., 2019). Moreover, plant diseases associated with root is a problem for seedling growth and the seedling production system. Those problems affect the health and quality of plant seedlings, though affect plant growth when planted under the field conditions. The high efficiency of seedling production is another option to increase crop yield and the value of the plant. Since the germinate phase of the seedling is the essential first phase, which affect growth and vigor in the next phase. The high quality of seedlings is therefore, necessary for economic crop production.

Currently, artificial light technology is being developed to control the amount of light that plants require. Usually, artificial light was used to plant crops in a place with not enough light. Artificial light has been used for the stimulation of plant growth by supplementing a specific wavelength of light to plants. The principle of this technology is to regulate light quantity in terms of time, wavelength, and light intensity, which plants use for nutrient synthesis. The general lamp used in the planting of closed systems is a Light-emitting diode (LED) lamp. The LED lamp provided, low-temperature, no disadvantage on plant growth, and can adjust several wavelengths. Many previous reports showed that cropping under some frequency of each wavelength, such as providing the appropriate ratio between red and blue light appropriate ratio can stimulate seed germination, growth, productivity, and product quality of many plant species (Wojciechowska et al., 2015; Lian, 2002; Yao et al., 2017; Chen et al., 2014; Chen et al., 2017; Piovene et al., 2015; Simlat et al., 2016; Y. Xu et al., 2016). In addition, LED light can stimulate the plant's resistance to plant pathogens (H. Xu et al., 2017). The wavelength of the LED lamp has affected the quality of the seedling. The red leaves lettuce under the red light shows higher leaves area and fresh weight (Johkan et al., 2010).

Currently, plants-associated microbe with symbionts had shown the potential to improve plant growth. The beneficial microorganisms can be applied in the agricultural system act as biofertilizers, bioherbicides, biopesticides, and biocontrol agents (Elizabeth Temitope Alori et al., 2018). Using microbes or their metabolites increases the plant nutrient uptake, yield, controls pests, and mitigates plant stress responses (Trivedi et al., 2017). Maize growth has been reported to increase due to stimulation with bacterial species (Elizabeth T. Alori et al., 2019). The application of beneficial microorganisms in organic cultivation demonstrated the increasing yields of vegetable and fruit species and which, agriculture production are sustainable when

using plant growth-promoting rhizobacteria in system production (Tarro, 2017). Additionally, arbuscular mycorrhiza fungi (AMF) are the microbe that is considered as an natural biofertilizers, because the AMF provides the host with water, nutrients, and pathogen protection, in exchange for photosynthetic products (Berruti et al., 2016). The mycorrhizal colonization enhances watermelon seedlings under drought condition through a stronger root system and greater protection of photosynthetic apparatus, a more efficient antioxidant system, and improved osmoregulation (Mo et al., 2016). Besides, AMF enhanced ATP synthesis and provided the energy for plant cell activity (Jia et al., 2019) and Commatteo et al., (2019) found that the tomato co-inoculation AMF and *Trichoderma* can increase the tomato growth. Also co-inoculated AMF and *Trichoderma* showed the positive effect to tomato seedling growth (Chliyeh et al., 2014). However, there was no study take advantage of the combination of LED light with microbes for plant growth promotion.

Therefore, LED light technology and beneficial microorganisms may apply to seedling production systems to enhance the health of seedlings. The plants selected from economic crops in Thailand were used in this study, and were tested for the optimum condition for high qualitative seedling production using LED light technology in combination with beneficial microorganisms.

1.2 Research objective

1.2.1 Main objective

To develop appropriate technology by incorporating the benefit of LED light, Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhiza Fungi (AMF) on the quality of economic seedling production.

1.2.2 Specific objective

a) To determine the optimum LED light condition that can enhance plant seedling production.

b) To investigate the influence of PGPR and AMF on seedling growth.

c) To demonstrate the efficiency of LED light, PGPR, and AMF to enhance plant seedling production.

d) To examine the efficiency of seedlings produced to the yield production.

e) To investigate the mechanisms of plant seedlings' response to LED light.

CHAPTER II LITERATURE REVIEW

2.1 Role of artificial light source on plant growth and development

Light is an electromagnetic wave caused by nature (Source: sunlight) or may caused by man-made (Source: artificial light). The appropriate light environment is essential for optimal plant growth and development (Dutta Gupta & Agarwal, 2017). However, the unsuitable light negatively affects the growth and development of plants because the light is one of the key environmental factors that have a major impact on plant architecture (Abidi et al., 2013). The application of artificial light is used supplementally in places where light is not enough, such as plant cultivation indoor or the events that accelerated the plant growth and yield. The common artificial light source used include incandescent lamps (ILs), fluorescent lamps (FLs), high-pressure mercury lamps (HPMLs), high-pressure sodium lamps (HPSLs), and metal-halide lamps (MHLs). Those artificial light sources are containing with disadvantages in generating heat, big size, and are energy-consuming. One of the alternative light sources is Light Emitting Diode (LED) since LED lighting systems contain several unique advantages including the ability to control the spectral composition, a small mass and volume, durability, long operating lifetimes, wavelength specificity and narrow bandwidth, relatively cool emitting surfaces, minimum heating, and photon output that is linear with the electrical input current (K.-H. Lin et al., 2013). Those features are appropriate to apply for plant cultivation. The influence of light affects plant throughout the plant life cycle such as seedgermination, stem elongation, and flowering. Moreover, the spectrum and intensity of light affect changes in plant morphology, physiology, biochemistry, and transcriptional expression (Ren et al., 2018). In terms of light quality, both red and blue lights alter plant architectural development. Effects of light are observed on processes and phenomena throughout the plant life cycle, including seed germination, stem elongation, leaf expansion, the synthesis of photosynthetic, hormone synthesis, and flowering. (Inoue et al., 2008; Z. Li et al., 2021; Modarelli et al., 2020; OuYang et al., 2015; Solano et al., 2020).

2.1.1 Plant photosynthesis process

Plant photosynthesis is the physicochemical process in plants. Plants use light energy to drive the synthesis of organic compounds. In plant photosynthesis, plants use light energy to make carbohydrates (Glucose: $C_6H_{12}O_6$) from the molecular of water (H_2O) and carbon dioxide (CO_2) and release oxygen gas (O_2) (Fig 2.1) (Singhal et al., 1999).



Figure 2.1 Equation of the plant photosynthesis (Zerrudo, 2020).

Plant photosynthesis contains two reactions including Light-dependent and Light-Independent reactions.

Light-dependent reactions

Light-dependent reactions this requires light energy to drive the electron, this reaction occurs in the chloroplast. The light was absorbed by pigment on the thylakoid membrane (such as chlorophyll, carotenoids, and xanthophylls) that contain in chloroplasts. Those plant pigments absorb primarily visible light (wavelengths from 400 to 700 nm) called photosynthetically active radiation (PAR) (Fig 2.2).

The combination of pigments on the thylakoid membrane is called the antenna complex. Plants pigments absorb light, then sent to the reaction center is chlorophyll a, subsequence chlorophyll transfers the electron to primally electron receptor. Working together between antenna complex reaction center and primally electron receptor called photosystem.



Figure 2.2 The electromagnetic spectrum (Pérez, Teixeira da Silva, & Lao, 2006).

The photosystem is divided into two groups by the type of pigment in the center of the reaction as a basis in the classification, including photosystem I (PSI) may call P700, and photosystem II (PSII) may call P680. Both the photosystems cause electrons to transfer in two ways non-cyclic electron transfer and cyclic electron transfer. Non-cyclic electron transfer is a process in which substances are used as electron receptor and electron transfer (e-) from water molecules. This process occurs within the thylakoid lumen. The electron is transferred to NADP+ which is in the stroma of chloroplasts. This phenomenon is causing a difference in the ion potential between the external and internal thylakoids, leading to the synthesis of ATP. The product of non-cyclic electron transfer is oxygen, NADPH, and ATP (Allen, 2003). Cyclic electron transfer occurs in the insufficient state of NADP+ within the chloroplast. Cyclic electron transfer is similar to non-cyclical electron-like nature, but the electrons that transfer from PSI to ferredoxin (Fd) occurred when NADP+ does not enough to receive the electron. Ferredoxin then sends electrons back to the plastoquinone (PQ), which transmits electrons to the cytochrome b6f complex, plastocyanin (PC), and back to the PSI (Fig 2. 3). Then ATP and NADPH were used in the Light-independent reactions to produce glucose and other organic compounds.



Figure 2.3 The Diagram of Photosynthetic Electron Transport. Photosystem I (PSI), Photosystem II (PSII), Cytochrome b6f complex (Cyt b₆f), plastocyanin (PC), Ferredoxin (Fd) and ferredoxin-NADP reductase (FNR). Cytochrome c6 (Cyt c₆) transfers electrons from the Cyt b₆f complex to PSI at a faster rate than observed for PC; plant ferredoxin-like protein (PFLP) (Adapted from Simkin, 2019).

Light-independent

Light-independent reactions, this process is fixing carbon dioxide to produce glucose and other organic compounds within the stroma of chloroplast called Calvin –Benson cycle. Light-independent reactions are separated into three main steps including carboxylation, reduction, and regeneration (Fig 2.4).

Carboxylation is carbon dioxide fixing to fused with Ribulose-1,5-bisphosphate (RuBP) by catalyzing Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo). The RuBP, a substance with five carbon atoms, reacts with carbon dioxide. A new compound with six carbon atoms is obtained. The emerging substance is unstable and decomposes to 2 phosphoglycerate (PGA) molecules, of which 1 PGA molecule has 3 carbon and 1 phosphate (Fig 2.5).



Figure 2.4 The process of carbon fixing in Calvin – Benson cycle (Bartee, Shriner, & Creech, 2017).



Figure 2.5 CO₂ activation by ribulose 1,5-phosphate as the first step in Ribulose-1,5bisphosphate carboxylase (RuBisCo) carbon fixation (Luca & Fenwick, 2015).

Reduction is a reaction in which the PGA molecule receives a phosphate group from ATP to become 1,3-bisphosphoglycerate. Then 1,3-bisphosphoglycerate is reduced to sugar with 3 carbon atoms called glyceraldehyde 3-phosphate(G_3P) or Phosphoglyceraldehyde (PGAL) by receiving electrons from NADPH (Fig 2.6).



Figure 2.6 Conversion of 3-phosphoglycerate into riose phosphate (Heldt & Piechulla, 2011).

Regeneration is the process of regenerating RuBP to obtain CO_2 again. To generate RuBP with 5 carbon atoms, it must be generated from PGAL which has 3 carbon atoms. This step requires ATP, so the balanced reaction is that 5 molecules of PGAL (30 carbon atoms) are needed to form 3 molecules of RuBP (Fig 2.7).



Figure 2.7 The Regeneration process of RuBP (Tymoczko, Berg, Jr, & Stryer, 2018).

2.2 Light- emitting diode (LED)

LED light is a common lamp seen in electronic devices such as digital clocks, remote control, electrical equipment page or even traffic light. LED lamps are small size bulbs, the LED lamps different from the incandescent lamp because there is no incandescent inside the lamp, therefore the LED lamp does not generate heat. Light is brightly generated by the movement of electrons inside the semiconductor. The semiconductor is the same material used to make transistors. LED lamps can emit light with a single frequency and continuous phase. The color spectrum of red, blue, and white LED will be given the spectrum light range by similar with the most light from the sunlight, which the red and blue light districts being used in the chlorophyll field of the plant. The red light wavelengths are 640 to 680 nm and the blue light wavelengths are 430 to 450 nm, they are necessary spectral for

plant development. The plant photoreceptor of red light is called phytochrome including phyA, phyB, phyC, phyD, and phyE, and the photoreceptor of blue light has many kinds including cryptochromes (CRY1 and CRY2), phototropin (PHOT1 and PHOT2) and LOV/F-box/Kelch-repeat protein (ZTL, LKP2, and FKF1) (Sun et al., 2020; Enderle et al., 2017; Yu et al., 2010).

Xu et al., 2017 reported that plants grown under artificial light were able to decrease the virulence of *Botrytis cinereal*; it is a causative agent of leaf disease when tomatoes grew under purple and blue light. Moreover, red and blue light reduce the size of the wound from infection by around 32.08 and 36.74%, respectively, while red light decreases superoxide anion oxidation and stimulates various enzymes in leaves to more resistant to plant diseases such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). Also LED light affects changes gene expression levels and enzyme activity in the plant such as Ribulose bisphosphate carboxylase large chain (*rbcL*), ferredoxin (*fdx*) and ATP synthase subunit beta (*atpB*) genes, and superoxide dismutase (SOD), ascorbate peroxidase (APX) and Catalase (CAT) enzyme activities (Ren et al., 2018; Wu et al., 2014).

2.3 The role of red (R) and blue (B) light on plant growth and development2.3.1 Role of red (R) light on plant

The red light that illuminated on plants was responded by phytochrome act as photoreceptors. The phytochrome mediates in the adaptation of plant growth and development by red light (J. Li et al., 2011; Rockwell et al., 2006; Xu et al., 2015). Manny studies reported the red light influence on plant growth and development. Hoenecke et al., (1992) revealed that expose of red light alone showed negative effect on plant biomass of lettuce, spinach, and radish than composition of red and blue or white light. Similary, the *Cordyline australis, Ficus benjamina* and *Sinningia speciosa* performed lower dry weight under red light (Zheng & Van Labeke, 2017). While, Y. Li et al., (2020) found that red light showed a negative effect on biomass accumulation, CO₂ assimilation and photosystem II (PSII) electron transportation when compared to the blue light or blue combination with red light. In addition, the red light alone could enhance growth and antioxidant

activity of *Rubus hongnoensis*, it was resulting on promote the phenolic compound formation when compared to other light conditions. However, the red light results in promote of long plant stem such as *Rubus hongnoensis, Arabidopsis thaliana* and *Solanum lycopersicum* (Oh, Yoon,, 2021; Spaninks et al., 2020). Similar results were reported the red light could promote the plant height, root development, leaf extension of apple seedlings. Moreover, the resulted of RNA-seq analysis of apple seedlings suggested that the plant hormone signal transduction can be induced by red light. Whereas, the chlorophyll a, b contents and net photosynthetic rate were significantly reduced (*Z*. Li et al., 2021). Moreover, the red light could promote the phytohormone in plant seedling to induce root development (Alallaq et al., 2020; Kumari & Panigrahi, 2019). In contrast, the red light inhibits the indole-3-acetic acid (IAA) production in norway spruce seedling. While the gibberellin (GA) was increased and promote the long stem when plant illuminated under the red light (OuYang et al., 2015).

2.3.2 Role of blue (B) light on plant

Blue light has several effects on plant development. The photoreceptor of blue light are phototropin and cryptochromes. They mediate the regulating blue light responses of plant growth and development (Inoue et al., 2008; Kang et al., 2008; C. Lin, 2002; C. Lin & Shalitin, 2003), including stomatal opening, suppressing hypocotyl elongation, photomorphogenesis, photosynthesis, phenolic compounds, and plant hormones regulations. It has been reported that the blue could regulate the stomatal opening (Inoue & Kinoshita, 2017; Suetsugu et al., 2014; Zeiger & Hepler, 1977). The controlling of stomata aperture is necessary for plant growth and plant survival because the CO₂ assimilation and transpiration were induced via stomata (Toh et al., 2018). In addition, Izzo et al., 2020 found that when tomato seedling was treated under pure blue light results in shorter of hypocotyl compared to red, white, and red combined with blue light. While the plants grown under blue light was shorter of stem and small life, whereas it was the highest levels of light harvesting pigments (Dieleman et al., 2019). As a Ficus benjamina the blue light increased the stomatal conductance and the role of leave structure (Zheng & Van Labeke, 2017). The blue light or blue combination with red light could enhanced the plant biomass and CO₂ assimilation of chili seedlings. Also, the leaf structures were

thickened and rich in Calvin cycle-related enzyme activity such as RuBisCo, fructose-1, 6-bisphosphatase and glyceraldehyde-phosphate dehydrogenase and ribulose-1, 5bisphosphate (Y. Li et al., 2020). In addition, the blue light has effect on the photosynthetic related genes, such as the *psbA*, *psbB*, *psbC*, *psbD*, *psaA*, and *psaB* were significantly up-regulated when cucumber seedlings were grown under blue light compared to white light (Miao et al., 2016). OuYang et al., (2015) found that blue light enhances the indole-3-acetic acid (IAA) formation and it can induce the up-regulated of auxin gene and secondary biosynthesis genes in norway spruce seedling.

2.3.3 Role red (R) combination with blue (B) light

The red and blue lights have widely known that it is important for plants growth and development. However, only red or blue lights alone may not enough to enhance high quality seedling. The monochromic of red light was treated with plant usually promoted plant extension and lead to long stem and thin leaf. In contrast, blue light inhibits plant extension cause dwarf phenotype. The combination of red with blue light may be better to improve quality of plants seedling. As a F. benjamina was planted under blue light and blue combined with red light resulted in the maximum quantum yield (Fv/Fm) and quantum efficiency (Φ PSII) were increased (Zheng & Van Labeke, 2017). The combined red and blue light could promoted Salvia miltiorrhiza growth greater than monochromic red and blue light, the results suggested that the rich of phenolic acid and high plant weight were induced by combined red and blue light (Zhang et al., 2020). The tomato seedling was treated under combined of red and blue light could promote plant fresh weight and dry weight greater than red light alone and more ratio blue light (Son et al., 2018). In addition, the different light quality effect on changing of biomass, photosynthetic related genes and enzyme activity. Especially, combination of red and blue light including ribulose bisphosphate carboxylase large chain (RBCL), ferredoxin (FDX), ATP synthase subunit β (ATPB) (C), isocitrate dehydrogenase (IDH), fructose-bisphosphate aldolase (FBA), and nucleoside diphosphate kinase 1 (NDPK 1). Moreover, the antioxidant enzyme was as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were has changed in Cunninghamia lanceolata seedlings under different light quality (Ren et al., 2018). Also, the supplementation of mixture red and blue light could enhance biomass, root development, photosynthetic pigment. Moreover, photosynthetic capacity and photosynthate production were enhanced under combination of red and blue light (Wang et al., 2022).

2.4 Role of Plant Growth Promoting Rhizobacteria (PGPR)

In addition to LED lighting, many kinds of microorganisms can enhance seed germination and plant health. Plant Growth Promoting Rhizobacteria (PGPR) are group of microorganisms which the function of microorganisms affect plant via productions of phytostimulators, biofertilizers such as production of phytohormones, biological nitrogen fixation, phosphates solubilization and biocontrol (Fig 2.8) (Nadeem et al., 2013). The PGPR strains contain many mechanisms to enhance plant growth. Moreover, some species of microorganisms can control pest and induce the plant resistant to adverse environmental conditions (Bouillant et al., 1997; Fernández-Aparicio & Rubiales, 2010; Sharma et al., 2003). Recent studies have reported that the bio-fertilizer was used to promote plant growth in many plant species. The microorganisms including bacteria and fungi can promote plant growth (Ceratorhiza et al., 2009; Mastouri et al., 2010), in case of bacteria, such as Pseudomonas, Brevibacillus, Azospirillum, Actinobacteria and Firmicutes (Chowdhury et al., 2009; Oliveira et al., 2009; Naiman et al., 2009; Piromyou et al., 2011). Sharma et al., 2003 found that Ceratorhiza sp. UAMH 9847 could stimulate seed germination of *Platanthera praeclara* and activate growth of orchid. However, the previous study has limited in some groups, there is no currently reported about the effect of planting under mixture with LED light and microorganisms to increase the quality and health of plant seedling productions. Another important advantage of using biofertilizer in seedling culture is that these microorganisms will remain with root before move the plant to farm. These microorganisms will have a role to promote plants when plants in farms.



Figure 2.8 The role of PGPR to enhancing plant growth (Nadeem et al., 2013).

2.5 Role of Arbuscular mycorrhiza fungi (AMF)

Arbuscular mycorrhiza fungi (AMF) are one type of mycorrhiza, which symbiosis with plant by penetrate into the plant host cell. The AMF show many roles in plant such as plant disease resistant, drought resistant, salinity resistant and nutrient absorptions. The nutrient absorptions were found when associate with interaction between AMF and plants by exchange each other with the nutrients. Importantly, phosphorus compound is nutrient that AMF absorb mostly and deliver to plants. However, AMF is able to absorb other nutrients, such as nitrogen, potassium, calcium, magnesium, copper and ion compounds. These nutrients are absorbed through the protein on the membrane of AMF which has a specific function, such as Pi transport, NH_4 transport, NO_3 transport, amino acid (AA) transport and urea transport (Mohanta & Bae, 2015) (Fig 2.9). The mixture of mycorrhizal fungus and rhizobacteria could promote banana growth and nutrition. This report showed higher significant in fresh weight, aerial dry weight, shoot length and leaf area of banana (Rodrigues et al., 2018). The co-inoculation of AMF and plant growth promoting

bacteria (PGPB) could reduce stress and promote crop productivity under salinity condition. Moreover, those co-inoculation stimulate of some metabolites such as phenolic, proline, peroxidase, soluble sugar, and peroxidase enzyme activity, which some of this metabolites associate with resistant plant drought stress condition (Moreira., 2020; Behrooz et al., 2019). Additional AMF show the role on tomato growth when single inoculate with *Glomus intraradices, G. mossea* and co-inoculate both of them. The results demonstrated higher in shoot and root dry weights of inoculated plant, while value analysis of phosphorus in leaves found that plant contains high phosphorus concentrations when inoculated with *G. intraradices* and *G. mossea* (Taoheed, Ateka, & Losenge, 2018).



Figure 2.9 Scheme summarizing the main nutrient exchange processes in ectomycorrhizas (EM) and arbuscular mycorrhizas (AM) symbiosis (Bonfante & Genre, 2010).

CHAPTER III MATERIALS AND METHODS

3.1 Plant materials

To investigate the plant response under LED light, the five plant species including Tomato sweet girl (*Solanum lycopersicum* L.), Chili jindadang (*Capsicum annuum* L.), Melon cat697 (*Cucumis melon* L.), Chinese kale (*Brassica oleracea* L.) and Mustard green (*Brassica juncea* (L.) *Czern*.) were used in this study. The seeds were surface sterilized by washing in 95% ethanol for 10 second, then washed again with 3% of sodium hypochlorite (NaOCl), washed in sterilized water eight times, finally soaked in sterilized water for 12 hours (h). The seeds were germinated by seeds sown in the seed trays containing sterilized peat moss. After that seed tray of tomato and melon were placed under LED light for 14 days. While chili, mustard green, and Chinese kale were treated under LED light for 21 days.

3.2 Investigate the optimum LED light condition

The seed was placed under the mixture LED light red and blue lights to determine the optimum light conditions. The suitable light intensity of seedling was determined using a ratio of 50:50 percent (%) between red and blue light (at 50, 100, 200, 300, 400 and 500 µmole/m²/s), fluorescent light at 150 µmole/m²/s and the greenhouse as control condition. Then, the selected light intensity was used to find the specific light ratio for seedling grown. The light ratio was investigated under various ratio of LED light between red (R%) and blue (B%) light ratio (at R80:B20, R60:B40, R50:B50, R40:B60 and R20:B80). Then, the suitable light intensity and light ratio were used to find the specific light photoperiod for seedling growth. The seedling growth was examined under different photoperiods. The light photoperiods test was divided for two experiments, the first the seeds were treated under illumination of LED or fluorescent for 10, 12, 14 hours/day and the greenhouse condition as a control. The second, seeds were treated under illumination of LED or fluorescent for 14, 18, 20 and
24 hours/day in case of highest health index was found under artificial light

at 14 hour/day and the greenhouse condition. The optimum condition of each plant experiment was further used in the next experiment.

3.3 Ability of PGPR on seedling growth

To investigate the effect of PGPR strains on seedling growth, the bacterial strains including Bradyrhizobium sp. SUTN9-2, Pseudomonas sp. SUT19, Bacillus velezensis S141, Bacillus megaterium A20 and four bacterial isolates from root of the plant seedling including tomato (Shinella sp. Ch12 and Bacillus velezensis SD10), melon (*Pseudomonas aeruginosa* Cat697) and papaya tree (*Enterobacter* sp. 3D13) were used in the study. The PGPR characteristics was investigated including indole -3-acetic acid (IAA) production (Sibponkrung et al., 2020), nitrogen fixation (screening by LG N-free medium agar), aminocyclopropane-1-carboxylic acid (ACC) deaminase production (Ali, Sandhya, & Venkateswar Rao, 2014), phosphate solubilization (Zeng, Wu, Wang, & Ding, 2017), and biocontrol as antagonistic bacteria (Yuttavanichakul et al., 2012). The high ability of the PGPR strains were determined by examining the seedling growth promotion under greenhouse condition. The sterilized-seeds were inoculated with PGPR strains. The PGPR inoculant was prepared by culturing in media are SUT19, S141, A20, Ch12, 3D13, Cat697, SD10 using Luria-Bertani (LB) medium and SUTN9-2 using yeast-mannitol (YM) medium (at 150 rpm at 30 °C, 24 h or 5 days for SUTN9-2). The cell culture was centrifuged at 4,000 rpm for 10 mins and discarded supernatant. Then cell pellet was washed using 0.85% NaCl twice and adjusted the cell density at OD600 as 1.0. Finally, the cell suspension was 10-fold diluted using DI sterilized water as diluent. After that seed sterilized was inoculated with cell suspension (by soaking the seed in the cells suspension for 10 minutes). Then the seeds were planted in the seeds tray containing sterilized peat moss and incubated in the darkroom for 48 hours, then placed under greenhouse. Then 2 strains of PGPR that can promote plant seedling growth were selected. Subsequently, 2 strains of PGPR selected were used to test on seedling under the optimum light condition obtained from this study. The seeds with non-inoculated was used as a control. The optimum condition of each experiment was used further in the next experiment.

3.4 Ability of AMF to seedling growth

Plant seedling was produced under optimum condition of LED light co-inoculated with PGPR were used in this experiment. Plant seedlings without inoculation were used as control produced under greenhouse. Plant seedling was inoculated with 500 spores of AMF (*Rhizophagus irregularis.*) then planted in Leonard jar containing with vermiculite sterilized (1 plant/pot, for 4 replication) using Hoagland solution (Half-strength phosphate) applied from Kaur et al., (2016). Then placed under greenhouse. The growth rate of tomato, melon, capsicum seedling was measured from plant height, leaves number, stem diameter and chlorophyll content at 10 days after planted in the Leonard jar. Then, the plant height, stem diameter, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, total fresh weight, total dry weight, chlorophyll content using SPAD meter, and root colonization describe by Trouvelot et al., 1986 were measured at 30 days after plant in the Leonard jar.

3.5 Determination of optimum growth condition for seedling production

The sample of the seedling was verified the plant growth at 14 days-old for tomato and melon, while 21 days-old for chili, mustard green and Chinese kale after planting. The seedling was investigated the optimum growth condition with many parameters including plant elongation; the shoot height, stem diameter and root length. The shoot fresh weight, root fresh weight, root dry weight and shoot dry weight were weighted, while leave area was measured with leaf area meter (10 plant/replication for 4 replications), chlorophyll content was measured by chlorophyll Meter (SPAD). The root shoot ratio (Root dry weight/Shoot dry weight) and health index was followed accredited to Fan et al., 2013.

3.6 Determine the ability of seedling produced to the yield production

The tomato and Chinese kale seedling were used as plant model to investigated the productivity. The seedlings were produced under the optimum light conditions and greenhouse were transferred to experimental field (The soil nutrients: pH 7.12, OM 0.63%, EC 0.1397 ms/cm, N 0.032%, P 83.35 mg/kg, K 184.10 mg/kg,

Ca 4,242.5 mg/kg, Mg 486 mg/kg, Fe 62 mg/kg, Zn 2.95 mg/kg, Cu 7.65 mg/kg, Mn 13.05 mg/kg, the 5 kg of filter cake and 2.5 kg of organic fertilizer were added to the field for a square meter). The tomato seedling was planted for 3 replications (15 plants/ replication, distance 50*50 cm), while the Chinese kale was planted for 3 replications (40 plants/ replications, distance 30*30 cm). Both of plant cultivars were managed by nitrogen fertilizer (0.5 g /plant for every a week) and pesticides (according to product instructions) for growth and prevent the plant disease, respectively. The chemical fertilizer and pesticides were used in the right amount of each plant cultivar. The tomato was recorded the results including fruit number, fruit weight when ripen fruit at 76 day-olds and continue until 30 days (harvested for 7 times, period 3–5 days/times). The Chinese kale was recorded the results including fresh weight and dry weight when 50 days-olds.

3.7 Detection of H₂O₂ accumulation and antioxidant enzyme activities

The tomato seedling was selected to study the H_2O_2 localization and antioxidant enzyme activities. The tomato seedling at 14 day-olds was treated under optimum LED light for 3 h and greenhouse as control. Then leaves were cut and soaked in the 1 mg/ml of DAB (3,3'-Diaminobenzidine) solution (pH 3.8) for 12 h under darkness. Subsequently, leave was boiled in 95% ethanol for 10 mins and examined the H_2O_2 on leaves (Kumar, Yusuf, Singh, Sardar, & Sarin, 2014). The superoxide dismutase (SOD) activity was investigated along with method described by Muneer et al., 2016.

3.8 Gene reference selection and primers design

The tomato seedling was selected to study the relative gene expressions. Genes related to photosynthesis (Including: *rbcL*, *rcbS*, *atpB*, *fdx*, *psbA* and *psbB* genes) of tomato were investigated the relative expression level. The genes reference (GCA_000188115.3) was obtained from NCBI database for design the specific primers. The primers design was performed using Snap Gene Viewer 5.1.4.1 and obtained from study of Wu et al., 2014 and Guo et al., 2020 (Table 3.1).

Primer	Sequence (5'- 3')	Reference		
Actin	F : GAAATAGCATAAGATGGCAGACG	(Cup of al. 2020)		
	R : ATACCCACCATCACACCAGTAT	(Guo et al., 2020)		
rbcL	F : CTGCGAATCCCTCCTGCTTA			
	R : CCAACAGGGGACGACCATAC			
rcbS	F : TGAGACTGA GCACGGATTTG	(Wu et al., 2014)		
pabA	R : TTTAGCCTCTTGAACCT CAGC			
	F : CCGTAAAGTAGAGACCCTGAAAC			
pabB	R : TGGATG GTTTGGTGTTTTGATG			
	F : CCTATTCCATCTTAGCGTCCG			
	R : TTGCC GAACCATACCACATAG			
аtpВ	F : TGGGCGGTTTCGTAATGTTC			
	R : GTACCCGCAGACGATTTGAC	This shuch (
fdx	F : GTGTGATTCATACTCAC <mark>CA</mark> GG	R		
	R : CACCTGACCATTCTCAATTACAG			

 Table 3.1
 The primer sequence of the tomato on photosynthetic related genes.

3.9 Preparation of plant sample for RNA extraction

The tomato seedling was plant under optimum LED light (At 200 µmole/m²/s, R60:B40, 20 h/D) at 14 day-olds. Then the tomato seedling was treated under optimum LED light for 3 hours and 4 leaves was cut and extract the total RNA, the tomato seedling was planted under greenhouse at 14 day-olds and was exposed by sunlight for 3 hours as a control. The leave was ground in liquid nitrogen using a mortar and pestle to make the powder. After that the sample powdered for 100 mg was quickly transferred into 1.5 ml tubes for total RNA extraction. Total RNA extraction was extracted using FavorPrep Plant Total RNA Purification Mini Kit followed the manufacturers protocol.

3.10 Gene expressions analysis

The 500 ng of total RNA was converted to cDNA using iScriptTM cDNA Synthesis Kit. The cDNA sample was diluted for 10-fold by DI typeI for qPCR analysis (The component of qPCR for 10 μ l reaction: 5 μ l Luna® Universal qPCR Master Mix, 0.1 μ l of forward primer (10 uM), 0.1 μ l of reward primer (10 uM), 1 μ l of template, and

3.8 µl nuclease-free water.). The relative gene expressions (*rbcL, rcbS, atpB, fdx, psbA* and *psbB*) were detected using qPCR method and calculated using Applied Biosystem, QuantStudio Design (The condition: initial denaturation at 95°C for 5 minutes, (denaturation at 95°C for 30 seconds, and extension at 95°C for 30 seconds for 40 cycles)). Relative gene expression was analyzed by the comparative Ct method (- $\Delta\Delta$ CT) and actin (*ACT*) (Table 3.1).was used as the control to normalized of qRT-PCR results (Greetatorn et al., 2020).

3.11 Statistical analysis

Data in the experiment were resolved in elements as mean values and standard deviation with SPSS software (SPSS version 26.0 Windows; SPSS Inc., Chicago, IL) by Duncan's multiple range test and independent sample t-test (For enzyme activity and gene expression) indicates P<0.05 (Duncan, 1995).



CHAPTER IV

RESULTS

4.1 Effect of light intensity on plant seedling growth

4.1.1 Effect of different light intensity on tomato seedling growth

The highest plant height of tomato seedlings was found under greenhouse conditions a significantly different when compared to fluorescent and LED light conditions. The tomato seedlings exposed to fluorescent and LED light showed significantly reduced plant height when compared to tomato seedlings grown under greenhouse condition (Fig 4.1a). Meanwhile, shoot fresh weight and total fresh weight were slightly increased, when planted under LED light at 300 and 400 µmol/m²/s. The tomato seedling was exposed under LED light at 300 or less than 200 µmol/m²/s and grown under fluorescent light performed decreasing in shoot fresh weight and total fresh weight (Fig 4.1c and g). In addition, root length, shoot dry weight, root fresh weight, root dry weight, and the total dry weight were increased when grown under LED light greater than 200 µmol/m²/s when compared to control (Fig 4.1b, d, e, f, and h). The tomato seedling treated under LED light of more than 100 µmol/m²/s was found not significantly different in stem diameter when compared to control. The stem diameter was reduced when it was exposed under LED light at 50 µmol/m²/s or fluorescent light when compared to control (Fig 4.1i). Also, under LED light at 50 µmol/m²/s, the leaf area was significantly reduced when compared to other treatments (Fig 4.1j). The chlorophyll content was higher under fluorescent or LED light at equal to or greater than 50 µmol/m²/s, and most of the chlorophyll content was found under LED light at 500 µmol/m²/s significantly different when compared to control (Fig 4.1k). While the root/shoot ratio was increased when the planted under LED light at 50 µmol/m²/s resulted in a significantly different when compared to the control (Fig 4.11). Then those previous results were used to calculate the health index of tomato seedlings and focusing on thehigh health index to define the optimum light intensity. The high health index was found under LED light intensity at 200, 300, 400 and 500 µmol/m²/s significantly

different when compared to control (Fig 4.1m). These results demonstrated that the LED light at 200 μ mol/m²/s resulted in the high strength of tomato seedlings. It did not differ with the light intensity at 300, 400, and 500 μ mol/m²/s. The phenotype of tomato seedling grown under different light intensity was depicted in figure 4.2.





Figure 4.1 The effect of light intensity on tomato seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), health index (m). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure 4.2 The phenotype of tomato seedling growth under different light intensity. Wash planting material (a), non-wash planting material (b).

4.1.2 Effect of different light intensity on melon, chili, mustard green, and Chinese kale seedling growth

The melon, chili, mustard green, and Chinese kale were examined under the various light intensity along with the condition used with the tomato seedling. The results showed that the melon grown under the LED light at 50 to 500 μ mol/m²/s resulting in significantly higher in health index when compared to control. The highest health index was found under LED light at 400 μ mol/m²/s, but it was not significantly different when compared to under LED light at 300 or 500 μ mol/m²/s (Fig 4.3a).

The chili health index was significantly higher when plants were exposed under LED light at 200 to 500 μ mol/m²/s when compared to control. The highest health index was found under LED light at 400 μ mol/m²/s, but it was not significantly different when compared to under LED light at 200 or 500 μ mol/m²/s (Fig 4.3b).

The highest health index of mustard green was found under LED light at 400 μ mol/m²/s and slightly reduced under 300 μ mol/m²/s and it was significantly higher than control (Fig 4.3c).

For the Chinese kale, the health index was significantly the highest in plant under LED light 400 µmol/m²/s when compared to other treatments (Fig 4.3d).

Therefore, in this study suggested that the LED light at 200, 300, 200, 300, and 400 µmol/m²/s were optimal light intensity for growing tomato, melon, chili, mustard green, and Chinese kale seedlings, respectively. These conditions were chosen for next experiments.



- Figure 4.3 The effect of light intensity on health index of plants seedling growth. Melon (a), chili (b), mustard green (c), and Chinese kale (d). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.
- 4.2 The effect of red (Red: R) and blue (Blue: B) light ratios on the plant seedlings growth

4.2.1 The effect of red and blue light ratios on the tomato seedlings growth

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The highest plant height of tomato seedling was found under the LED light ratio at R80:B20, while the light ratio at R20:B80 resulted in shorten plant height (Fig 4.4a). While, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, total fresh weight, total dry weight, stem diameter and the leaf area (Fig 4.4b and j) were increased by light ratio at R60:B40. However, changing in red light to R80:B20 or increasing the blue light ratio to R50:B50, results in slightly reduced of those parameters, but those parameters were greatly reduced under light ratio at R40:B60 and R20:B80. The seedlings grown under LED light at R50:B50 showed the highest chlorophyll content, while increasing the blue light ratio up to R40:B60 resulting in a slightly decrease of chlorophyll content. However, significantly reduce of chlorophyll content was found under light ratios R20:B80, R60:B40 and R80:B20 (Fig 4.4k). In terms of root/shoot ratio, it was found that seedlings grown under light ratio at R40:B60 showed a significantly reduced when compared to other treatments (Fig 4.4l). Then those previously results were used to calculate the health index of tomato seedlings and focusing on the high health index to define the optimum light ratio. The highest health index was found under light ratio at R60:B40, followed by the light ratio at R50:B50 (Fig 4.4m). The phenotype of tomato seedling grown under different light ratio was depicted in figure 4.5.





Figure 4.4 The effect of light ratio on tomato seedling growth, shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), health index (m). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure 4.5 The phenotype of tomato seedling growth under different light ratio. Wash planting material (a), non-wash planting material (b).

4.2.2 The effect of red and blue light ratios on the melon, chili, mustard green, and Chinese kale seedlings growth

The melon, chili, mustard green, and Chinese kale were examined the light ratio along with the condition used with the tomato seedling and only health index was focused. The highest health index of melon was found under light ratio at R60:B40, while slightly reduced under light ratio at R50:B50, R40:B60, and R20:B80. However, health index was significantly reduced under light ratio R80:B20 when compared to other treatment (Fig 4.6a).

The chili health index was significantly the highest when planted under light ratio at R50:B50 when compared to other treatments (Fig 4.6b).

The health index of mustard green was the highest under light ratio at R50:B50 and slightly reduced under light ratio at R60:B40 or R40:B60 (Fig 4.6c).

In addition, the Chinese kale was significantly the highest on health index when plants were illuminated under light ratio R60:B40. The health index was slightly reduced when exposed under light ratio R50:B50 and significant higher than light ratio at R80:B20, R40:B60, and R20:B80 (Fig 4.6d).

These results indicated that the ratio of red light and blue light at R60:B40, R60:B40, R50:B50, R50:B50, and R60:B40 were the optimum light ratio that could enhance the growth and vigor of tomato, melon, chili, mustard green, and Chinese kale seedlings, respectively. These results demonstrated that the ratio of red light and blue light have plant variety specific and these conditions were chosen for next experiment.

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Figure 4.6 The effect of light ratio on health index of different plants seedling growth. Melon (a), chili (b), mustard green (c), and Chinese kale (d). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.

4.3 Effects of light photoperiod on tomato seedlings growth

4.3.1 Effects of light photoperiod on tomato seedlings growth at 10-14 hours/day

The effect of the period of lighting on tomato seedlings was found that a significantly reduce plant height when seedlings were planted under fluorescent light for equal to or greater than 12 h/D or under the LED light at equal to or greater than 10 h/D (Fig 4.7a). While the root length (Fig 4.7b), shoot fresh weight (Fig 4.7c), root fresh weight (Fig 4.7d), shoot dry weight (Fig 4.7e), total fresh weight (Fig 4.7g), total dry weight (Fig 4.7h), stem diameter (Fig 4.7i), and leaf area (Fig 4.7j) were significantly increased when planted under LED light at equal to or greater than 10 h/D when compared to the control. The total fresh weight and root dry weight were significantly different when growing tomato seedlings under LED light at equal to or greater than 12 h/D when compared to the control. The chlorophyll content was slightly increased when grown under fluorescent lighting at 10 and 12 h/D and significantly increased when tomato seedlings were grown under fluorescent light at 14 h/D or under LED light at equal to or greater than 10 h/D (Fig 4.7k). However, the root/shoot ratio was reduced when tomato seedlings were grown under the fluorescent light at or more than 10 h/D and under LED light at 10-12 h/D While the root/shoot ratio was significantly increased when grown under LED light at 14 h/D when compared to control (Fig 4.7l). Then those previously results were used to calculate the health index of tomato seedlings and high health index was found under LED light at equal to or greater than 10 h/D when compared to control. The higher health index was found under LED light at equal to or greater than 10 h/D when compared to control. The highest health index was found under LED light at 14 h/D a significantly different when compared to other treatments (Fig 4.7m). The phenotype of tomato seedling grown under different light photoperiod was depicted in figure 4.8.







Figure 4.7 The effect of light photoperiod on tomato seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), health index (m). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.

4.3.2 Effects of light photoperiod on melon, chili, mustard green, and Chinese kale seedlings growth at 10 - 14 hours/day

The melon, chili, mustard green, and Chinese kale were examined the light photoperiod along with the condition used with the tomato seedling. The highest health index of melon, chili, and mustard green were found under LED light at 14 h/D a significantly different when compared to control. While reduce the light photoperiods shorter than 14 h/D the health index was significantly reduced (Fig 4.9a, b, and c).

However, the highest health index of Chinese kale was found under LED light at 12 h/D significantly different when compared to control. When increase or reduce from LED light at 12 h/D the photoperiod, the health index was significantly reduced (Fig 4.9d). These results indigested that the light photoperiod at 12 h/D was the optimum photoperiod for Chinese kale seedlings. While the light photoperiod at 14 h/D was the optimum photoperiod for tomato, melon, chili, and mustard green, respectively. Consequently, when the photoperiod at14 h/D was applied to tomato, melon, chili, and mustard green planting was further investigated the photoperiod in a longer time than 14 h/D in the next experiment.



Figure 4.8 The phenotype of tomato seedling growth under different light photoperiod. Wash planting material (a), non-wash planting material (b).



Figure 4.9 The effect of light photoperiod on health index of different plants seedling growth. Melon (a), chili (b), mustard green (c), and Chinese kale (d). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.

4.3.3 Effects of light photoperiod on tomato seedlings growth at 14 - 24 hours/day

The tomato seedling, the plant height was shorter when plants were illuminated under fluorescent light at 18 h/D or LED light at equal or greater than 14 h/D when compared to the control (Fig 4.10a). While the root length was significantly increased when grown under all fluorescent light and LED light treatments (Fig 4.10b). Also, the shoot fresh weight (Fig 4.10c), root fresh weight (Fig 4.10d), roots dry weight (Fig 4.10f), total fresh weight (Fig 4.10g), total dry weight (Fig 4.10h), stem diameter (Fig 4.10i), leaf area (Fig 4.10j), chlorophyll content (Fig 4.10k), and root/shoot ratio (Fig 4.10l) were increased when grown under fluorescent light at 18 h/D or LED light at equal or greater than 14 h/D when compared to control. Then those previously results were used to calculate the health index of tomato seedlings

and the high health index to define the optimum light photoperiods. It was found that the tomato seedlings grown under LED light at 24 h/D showed the highest health index, but did not significantly different with under LED light at 20 h/D (Fig 4.10m). These results illustrated that the tomato seedlings were grown under LED light at 20 h/D resulting in high strength being significantly different when compared to control. While increasing the light photoperiod more than 20 h/D did not affect the health index (Fig 4.10). The phenotype of tomato seedling grown under different light photoperiod at 14–24 h/D was displayed in figure 4.11.





Figure 4.10 The effect of light photoperiod at 14–24 h/D on tomato seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh

weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), health index (m). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure 4.11 The phenotype of tomato seedling growth under different light photoperiod at 14-24 h/D. Wash planting material (a), non-wash planting material (b).

4.3.4 Effects of light photoperiod on melon, chili, and mustard green seedlings growth at 14 - 24 hours/day

The melon, chili, and mustard green were examined the light photoperiod at 14-24 h/D along with the condition used with the tomato seedling.

The results showed that the highest health index of melon was found when plant under LED light at 20 h/D. While the health index was slightly reduced under fluorescent and LED light at 24 h/D a significantly different when compared to control. However, planting the melon under LED light at equal or greater than 14 h/D or fluorescent light at equal or greater than 18 h/D the health index was significantly different when compared to control (Fig 4.12a).

As a chili, the highest health index performed under LED light at 24 h/D but was not significantly different when compared to under LED light at 18 h/D (Fig 4.12b).

For the mustard green, the highest health index was found under LED light at 18 h/D significantly different when compared to other treatments (Fig 4.12c). Therefore, an LED light duration of 20 h/D was the optimum photoperiod to tomato and melon seedling growth, while the photoperiod of chili, and mustard green were optimized at 18 h/D. The optimum light conditions for plant seedling growth were demonstrated in table 4.1. In this study, the optimal light condition for each variety was used in the next experiment.



Photoperiod

Figure 4.12 The effect of light photoperiod at 14-24 h/D on health index of different plants seedling growth. Melon (a), chili (b), and mustard green (c). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 05$.

	Light condition					
Plant seedling	Light intensity	Light ratio	Light photoperiod			
	(µmol/m²/s)	(%)	(h/D)			
Tomato	200	R60:B40	20			
Melon	300	R60:B40	20			
Chili	200	R50:B50	18			
Mustard green	300	R50:B50	18			
Chinese kale	400	R60:B40	12			

 Table 4.1 The optimum light condition for plant seedling growth.

4.4 The effect of PGPR on the plant seedlings growth

4.4.1 The effect of PGPR on the tomato seedlings growth

The inoculation of *Bradyrhizobium* sp. SUTN9-2, *Pseudomonas* sp. SUT19, *Bacillus megaterium* A20, *Shinella* sp. Ch12, and *Bacillus velezensis* SD10 with tomato seeds were significantly increased in plant height, while inoculation of *Bacillus velezensis* S141 with the tomato seeds did not affect to plant height. However, the tomato seeds were inoculated with *Enterobacter* sp. 3D13 or *Pseudomonas aeruginosa* Cat697, the tomato seedlings had significantly reduce plant height when compared to the control (Fig 4.13a). In addition, the root length was slightly increased when inoculated the SUTN9-2 or SD10. When Cat697 was inoculated with tomato seeds resulted in significantly reduced root length (Fig 4.13b). However, the shoot fresh weight (Fig 4.13c), shoot dry weight (Fig 4.13e), root dry weight (Fig 4.13f), total fresh weight (Fig 4.13g), total dry weight (Fig 4.13h), stem diameter (Fig 4.13i), and leaf area (Fig 4.13j) were increased when inoculated SUTN9-2, A20, and SD10 a significantly different when compared to control. While

inoculated of PGPR on other treatments, those results did not differ when compared to control or even results in a significantly reduced.

In addition, the results showed that the inoculation of SD10 with the tomato seedlings resulted in a significantly increased in chlorophyll content. While 3D13 and Cat697 were significantly reduced chlorophyll content when compared to control (Fig 4.13k). However, the inoculation of PGPR with tomato seeds did not affect to the root/shoot ratio (Fig 4.13l). Then those previous results were used to calculate the health index of tomato seedlings and the high health index to find PGPR strains that could promote seedling growth. The results showed that inoculation of SUTN9-2 with tomato seeds performed the highest in health index, followed by the inoculation with SD10 or A20 significantly different when compared to the control. These results showed that SUTN9-2, SD10, and A20 could promoted the tomato seedlings growth (Fig 4.13). The phenotype of tomato seedling inoculated with PGPR was shown displayed in figure 4.14.









Figure 4.13 The effect of PGPR on tomato seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), health index (m). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure 4.14 The phenotype of tomato seedling when inoculated with PGPR. Wash planting material (a), non-wash planting material (b).

4.4.2 The effect of PGPR on the melon, chili, mustard green and chinese kale seedlings growth

The melon, chili, mustard green and chinese kale were examined and selected the PGPR strains along with the condition used with the tomato seedling. The results showed that high health index of melon seedling was found when inoculated with SUTN9-2 with seed. Follow by non-inoculated PGPR and inoculated with SD10, 3D13 and A20. However, the health index was significantly reduced when inoculated with SUT19, S141, Ch12, and Cat697 (Fig 4.15a).

In case of chili seedling, the health index was significantly higher when inoculated with SUTN9-2, SUT19, Ch12, SD10, S141, and A20, respectively when compared to control (Fig 4.15b).

For the mustard green seedling, the results showed that the health index was significantly the highest when inoculated with SUT19 or SD10 when compared to control. While inoculation of Cat697, the health index was significantly reduced (Fig 4.15c)

In addition, the chinese kale seedling showed the highest health index when inoculated with S141 with seeds, followed by the inoculation of SUT19, and SD10 significantly different when compared to control (Fig 4.15d). The results demonstrated that the PGPR could enhance plant seedlings by inoculation of PGPR with seeds under greenhouse conditions. In addition, two strains of PGPR that could promote seedling growth of each variety, including SUTN9-2 and SD10 were inoculated with tomato and melon, SUTN9-2 and SUT19 were inoculated with chili, SUT19 and SD10 were inoculated with mustard green, and SUT19 and S141 were inoculated with chinese kale. Therefore, the strains were selected and used to inoculate with plant seeds under artificial light in the next experiment.



Figure 4.15 The effect of PGPR on health index of different plants seedling growth. Melon (a), chili (b), mustard green (c), and chinese kale (d). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.

PGPR characteristic						
Strains	IAA	N ₂ -	ACC	P solu-	Biocontrol	
	production	fixation	deaminase	bilization	ыосоннос	
Bradyrhizobium sp. SUTN9-2 (JN578804.1)	+	+	+	-	-	
Pseudomonas sp. SUT19 (HQ230346)	+	+	+	+	+	
Bacillus velezensis S141 (AP018402.1)	+	-	+	-	+	
Bacillus megaterium A20 (MT597980)	-	-	+	-	+	
<i>Shinella</i> sp. Ch12 (ON342887)	-	+	+	+	-	
Bacillus velezensis SD10 (ON342885)	+	+	+	-	+	
Enterobacter sp. 3D13 (ON342888)	+	-	+	-	+	
Pseudomonas aeruginosa Cat697	_	_	+	_	+	
(ON342886)			,			

 Table 4.2
 The PGPR characteristics.

15.

* The + is positive results, symbol - is positive results.

4.5 Effect of inoculation of PGPR with plant under the optimum light condition on plant seedling growth

4.5.1 Effect of inoculation of PGPR with tomato under the optimum light condition on plant seedling growth

For the tomato seedling, the results showed that the short plant height was found when planted under fluorescent or LED light. Also, the PGPR inoculation with tomato seeds had no effect on the plant height (Fig 4.16a). While the shoot fresh weight (Fig 4.16c), shoot dry weight (Fig 4.16e), root length (Fig 4.16b), root fresh weight (Fig 4.16d), root dry weight (Fig 4.16f), total fresh weight (Fig 4.16g), total dry weight (Fig 4.16h), and leaf area (Fig 4.16j) were significantly increased when seedlings were planted under LED light. Whereas, those parameters were significantly reduced when plants were grown under fluorescent when compared to the control. Moreover, when inoculated with SD10 and grown under LED light, the root fresh weight (Fig 4.16d), root dry weight (Fig 4.16f), and the total dry weight (Fig 4.16h) was significantly increased when compared to tomato seedlings grown under LED light without inoculation. The bigger stem diameter was found in the tomato seedling inoculated with SUTN9-2 and SD10 under greenhouse condition and LED light and fluorescent light (Fig 4.16i). In addition, the chlorophyll content of tomato seedlings was increased when grown under fluorescent light and LED light when compared to greenhouse. While, inoculation of SD10 alone with tomato seedlings under greenhouse resulted in an increase in chlorophyll content similar to that under fluorescent light (Fig 4.16k). In term of root/shoot ratio, the result found that the inoculation of SD10 and planted under fluorescent light and both inoculation and non-inoculation under LED light, resulted in a high root/shoot ratio (Fig 4.16l). Then these previous results were used to calculate the health index of tomato seedlings. The high health index was used to determine the ability of PGPR strains on the promotion of seedling growth under artificial light. It was found that tomato seedlings grown under LED light performed high health index than seedlings grown under greenhouse and fluorescent light. However, the highest health index was found in the inoculation of SD10 with tomato seeds and grown under LED light. The results demonstrated that the light had a greater effect on the tomato seedlings

growth than the PGPR. However, SD10 was able to promote the tomato seedlings growth when used in combination with various light conditions, such as the shoot fresh weight, shoot dry weight and total dry weight were increased when SD10 was inoculated with tomato seedling (Fig 4.16). The phenotype of tomato seedlings grown when inoculated with PGPR under different light conditions was depicted in figure 4.17.





Figure 4.16 The effect of inoculation of PGPR and plant under the optimum light condition on plant seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter

(i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), health index (m). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure 4.17 Phenotype of tomato seedlings grown when inoculated with PGPR under different light conditions. Wash planting material (a), non-wash planting material (b).

4.5.2 Effect of inoculation of PGPR with melon, chili, mustard green and chinese kale under the optimum light condition on plant seedling growth

The melon, chili, mustard green and chinese kale were determined the ability of PGPG strains on the promotion of seedling growth under artificial light according to the treatment with tomato seedling. The inoculation of SD10 with melon seeds and plant under LED light results in the highest health index, follow by inoculation of SUTN9-2 with seeds or non – inoculated and plant under the LED light showed significantly different when compared to control (Fig 4.18a).

For the chili, the results showed that the health index was significantly the highest when inoculated with SUTN9-2 when compared to other treatments (Fig 4.18b).

The health index of mustard green was the highest when applied without inoculation under the LED light. However, it significantly reduced when seeds were inoculated with SUT19 or SD10 and planted under LED light when compared to non-inoculated and planted under LED light, but the health index was significantly higher than inoculated or non-inoculated and planted under fluorescent and greenhouse (Fig 4.18c).

In addition, the highest health index of chinese kale was found when inoculated with S141 and plant under LED light, followed by, non-inoculated or inoculated with SUT19 and planted under the LED light significantly different when compared to inoculated or non-inoculated under fluorescent and greenhouse (Fig 4.18d). The results demonstrated that the inoculation of PGPR with seedlings may either enhance or suppress them under artificial light.






- Figure 4.18 The effect of inoculation of PGPR and optimum light condition on health index of different plants seedling growth. Melon (a), chili (b), mustard green (c), and chinese kale (d). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.
- 4.6 Effect of PGPR inoculation with LED illuminated plant seedlings in combination with AMF on the growth of tomato seedlings under greenhouse conditions.
 - 4.6.1 Effect of PGPR inoculation with LED illuminated tomato seedlings in combination with AMF on the growth of tomato seedlings under greenhouse conditions at 10 and 30 days after seedling stage.

At 10 days after seedling stage, the results showed that the LED illuminated and LED unilluminated tomato seedlings inoculated SD10 or SD10+ *Rhizophagus irregularis* (AMF), the plant height was increased when compared to the seedlings without SD10 and AMF inoculation and control. While greater the stem diameter, chlorophyll content and leaves number were increased when the LED unilluminated seedling was inoculated with the SD10 or SD10+AMF when compared to control (Figure 4.19).



Figure 4.19 The effect of PGPR inoculation with LED illuminated tomato seedling in combination with AMF on the growth of tomato seedlings under greenhouse conditions at 10 days after seedling stage. Plant height (a), stem diameter (b), chlorophyll content (c), Leaf number (d). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.

At 30 days after seedling stage, the results showed that the plant height, shoot fresh weight, and total fresh weight were significantly increased when the tomato seedlings inoculated with SD10 or SD10+AMF when compared to the control. Especially, the LED unilluminated seedlings and inoculated with SD10+AMF results in a the highest of stem diameter, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, total fresh weight, total dry weight, and chlorophyll content. When AMF root colonization was determined, it showed that the highest colonization was found in the root tomato seedlings inoculated with AMF and grown under greenhouse conditions. While the root colonization was significantly reduced when the tomato seedling inoculated with AMF+SD10 and grown under greenhouse.

Also, the colonization of LED unilluminated tomato root seedling showed the same trend as in greenhouse. The results demonstrated that the inoculation of SD10 alone or SD10+AMF with tomato seedlings could promote the tomato seedlings growth. Especially, when inoculated the SD+AMF with LED unilluminated seedlings, it was able to grow well compared to other treatments (Figure 4.20).





Figure 4.20 The effect of PGPR inoculation with LED illuminated tomato seedling in combination with AMF on the growth of tomato seedlings under greenhouse conditions at 30 days after seedling stage. Plant height (a), stem diameter (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), chlorophyll content (i), root colonization (j), m%: intensity of the mycorrhizal colonization in the root fragments. Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure 4.21 The phenotype of LED illuminated tomato seedling when PGPR inoculation in combination with AMF on the growth of tomato seedlings under greenhouse conditions after seedling stage. Tomato plant at 10 day-olds after seedling stage (a), Tomato plant at 30 day-olds after seedling stage (b).

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4.6.2 Effect of PGPR inoculation with LED illuminated melon seedlings in combination with AMF on the growth of melon seedlings under greenhouse conditions at 10 and 30 days after seedling stage

At 10 days after seedling stage, the results showed that the LED illuminated melon seedling performed a statistically lower plant height than the seedlings planted in greenhouse conditions. While the seedling was inoculated with the SUTN9-2 or SUTN9-2+AMF showed no effect on the plant height. However, the LED illuminated melon seedling was resulted in significantly bigger stem diameter than the seedlings planted in greenhouse conditions. Whereas, when SUTN9-2 or

SUTN9-2+AMF were inoculated with LED illuminated seedling, the stem diameter was slightly reduced when compared to non-inoculated of LED illuminated seedling. The chlorophyll and the leaves number almost the same as other treatments (Figure 4.22).



Figure 4.22 The effect of PGPR inoculation with LED illuminated melon seedling in combination with AMF on the growth of melon seedlings under greenhouse conditions at 10 days after seedling stage. Plant height (a), stem diameter (b), chlorophyll content (c), Leaf number (d). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.

At 30 days after seedling stage, the results showed that the LED illuminated melon seedlings were inoculated with AMF was performed the highest plant height. While the LED illuminated melon seedling inoculated with SUTN9-2+AMF showed the highest stem diameter, shoot fresh weight, root fresh weight, and total fresh weight when compared with other treatments. The inoculation SUTN9-2 with LED

illuminated melon seedling performed the highest shoot dry weight and the total dry weight. The highest root dry weight was found in melon seedlings without inoculated when compared to other treatments. In addition, the chlorophyll content in all treatments showed almost likely the same. In terms of the root colonization of AMF in the melon roots, it was found that the melon seedlings that were inoculated with AMF had a percentage of AMF colonization of about 36-57% (Figure 4.23).





Figure 4.23 The effect of PGPR inoculation with LED illuminated melon seedling in combination with AMF on the growth of melon seedlings under greenhouse conditions at 30 days after seedling stage. Plant height (a), stem diameter (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), chlorophyll content (i), root colonization (j), m%: intensity of the mycorrhizal colonization in the root fragments. Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



- Figure 4.24 The phenotype of LED illuminated melon seedling when PGPR inoculation in combination with AMF on the growth of tomato seedlings under greenhouse conditions after seedling stage. Tomato plant at 10 day-olds after seedling stage (a), Tomato plant at 30 day-olds after seedling stage (b).
 - 4.6.3 Effect of PGPR inoculation with LED illuminated chili seedlings in combination with AMF on the growth of melon seedlings under greenhouse conditions at 10 and 30 days after seedling stage

At 10 days after seedling stage, the results showed that all of the treatments were similar in plant height and the number of leaves. While the inoculation of SUTN9-2, AMF or SUTN9-2+AMF with LED illuminated chili seedlings resulted in a significantly increased stem diameter when compared to the control. In

addition, the LED illuminated chili seedlings inoculated with SUTN9-2, AMF or SUTN9-2+AMF and without inoculated resulted in a significantly increased the chlorophyll content when compared to the control (Figure 4.25).



Figure 4.25 The effect of PGPR inoculation with LED illuminated chili seedling in combination with AMF on the growth of chili seedlings under greenhouse conditions at 10 days after seedling stage. Plant height (a), stem diameter (b), chlorophyll content (c), Leaf number (d). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.

At 30 days after seedling stage, the results showed that the LED illuminated chili seedlings inoculated with SUTN9-2, AMF or SUTN9-2+AMF and non-inoculated resulting in significantly increased in all of growth parameters when compared to control. While the chlorophyll content was similar in all treatments. The inoculation



of SUTN9-2 with chili seedlings resulted in a slightly reduce when compared to noninoculated chili seedling (Figure 4.26).



Figure 4.26 The effect of PGPR inoculation with LED illuminated chili seedling in combination with AMF on the growth of chili seedlings under greenhouse conditions at 30 days after seedling stage. Plant height (a), stem diameter (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), chlorophyll content (i), root colonization (j), m%: intensity of the mycorrhizal colonization in the root fragments. Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.





Figure 4.27 The phenotype of LED illuminated chili seedling when PGPR inoculation in combination with AMF on the growth of tomato seedlings under greenhouse conditions after seedling stage. Tomato plant at 10 day-olds after seedling stage (a), Tomato plant at 30 day-olds after seedling stage (b).

- 4.7 The ability of seedling produced under LED illumination and the yield production
 - 4.7.1 The ability of tomato seedling produced under LED illumination and the yield production

The harvesting yield from tomato seedlings produced in the greenhouse for 14 days showed that the fruit fresh weight yield was 953 g/plant or 19,068 kg/ha. While the fruit number was 72.2 fruits/plant. While the LED illuminated tomato seedlings inoculated with SD10 for 14 day and inoculated AMF before planting in the field showed that the fruit fresh weight yield was 1,105 g/plant or 22,119 kg/ha. While the fruit number was 76.9 fruits/plant. However, the seedlings grown under greenhouse conditions resulted in fruit weight and fruit number higher than LED illuminated tomato seedlings inoculated with SD10 and AMF during the 1st -3rd harvest times, while the 4th harvest time gave almost the same yield. In the 5th and 6th harvest times, it was found that LED illuminated tomato seedlings inoculated with SD10 and AMF showed the fruit weight was significantly higher than seedlings grown under greenhouse conditions, but the fruit number slightly increased in the 6th harvest time. However, at the 7th harvest time, it was found that fruit weight derived from inoculated with SD10 and AMF resulted in higher fruit weight and fruit number than these of seedlings grown under greenhouse condition, fruit weight and fruit number were increasing by 16% and 7.98%, respectively (Fig 4.28).





Figure 4.28 The ability of tomato seedling produced under LED illumination and the yield production. Tomato yield per plant (a), tomato yield per hectare (b), total fruit number (c), total yield follows harvesting periods (d), total fruit number follows harvesting periods (e). Mean and standard deviation are calculated from three replicates, the symbol of * indicate significant effect at $P \le 0.05$.

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4.7.2 The ability of chinese kale seedling produced under LED illumination and the yield production

The results showed that the chinese kale seedling produced in the greenhouse for 21 day was able to grow well when planted in the field, with the plant height and stem diameter of 17 cm and 24 mm, respectively. The fresh weight was 67 g/plant or 13,466 kg/ha, while the dry weight was 5.6 g/plant or 1,121 kg/ha. In regards to LED illuminated chinese kale seedlings inoculated with S141 for 21 days, the seedlings grew well when transplanted at the field. The plant height and stem diameters were 19 cm and 24 mm, respectively. The fresh weight was 77 g/plant or 15,327 kg/ha, while dry

weight was 6.35 g/plant and 1,270 kg/ha. The results demonstrated that the LED illuminated chinese kale seedlings inoculated with S141, resulted in fresh weight and dry weight increased by 13.82% and 13.29%, respectively when compared to the chinese kale seedling produced under greenhouse condition (Fig 4.29).

Figure 4.29 The ability of chinses kale seedling produced under LED illumination and the yield production. Plant height (a), stem diameter (b), fresh weight per plant (c), fresh weight per hectare (d), dry weight per plant (e), dry weight per hectare (f). Mean and standard deviation are calculated from three replicates, the symbol of * indicate significant effect at $P \le 0.05$.

4.8 The H₂O₂ accumulation and SOD activity assay and Photosynthetic genes expression in leaves

The hydrogen peroxide staining showed that the tomato leaves were dark brown when treated under LED light, while the tomato leaves from tomato seedling planted under greenhouse showed white (Figure 4.30a). The SOD activity was significantly increased about 1.8 folds in the tomato seedling grown under LED light when compared with the tomato grown under the greenhouse condition (Figure 4.30b). Moreover, the expression of *rbcL*, *fdx*, *atpB*, *psbA*, and *psbB* genes were significantly upregulated in tomatoes grown under LED light (2.6, 3.1, 2.0, 5.0, and 2.6 folds, respectively) when compared to tomatoes grown under the greenhouse condition. While the expression of the *rcbS* gene showed no significantly different compared to the tomato seedling grown under the greenhouse condition. This result can be concluded that the genes related to photosynthesis including *rbcL*, *rbcS*, *fdx*, *atpB*, *psbA*, and *psbB* were transcriptionally increased by LED light condition (Figure 4.30c).

Figure 4.30 The H₂O₂ accumulation and SOD activity assay and Photosynthetic genes expression in leaves. The hydrogen peroxide accumulation (a), superoxide dismutase activity assay (b), photosynthetic related gene

expression (c). Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.

CHAPTER V DISCUSSION

The red and blue light is the wave range that most plants require for photosynthesis. However, the light conditions received by plants change lead to effect on growth and development such as change in light intensity (Feng et al., 2019; Z. Ma et al., 2010; Modarelli et al., 2022; S. Olschowski, et al., 2016; T. Schumann et al., 2017), light ratio (Bartucca et al., 2020; P. Deram et al., 2014; Monostori et al., 2018; Naznin & Lefsrud, 2014; Q. Ying et al., 2021), and light photoperiods (J. Kang et al., 2013; Y. Xu et al., 2020). Whether the plants received less or excessive light affects the growth of plants as well. At the same time, when plants are exposed to optimum light conditions, they can grow well. In this experiment, tomato seedlings performed the highest plant height when planted in a greenhouse. While the 50:50 ratio of red and blue LED light at the light intensity of 50 to 500 µmol/m²/s and fluorescent light at 150 µmol/m²/ s resulted in a significantly reduced plant height (Fig 4.1). Previous reports have shown that blue light can trigger cryptochrome to take its active form and has an inhibitory effect on hypocotyl elongation (Dieleman et al, 2019; X. Yu et al., 2010). Ologundudu et al., 2013 reported that the plant height was reduced when exposed to high light intensity. While the plant height was increased in low light conditions, this may be due to limited photosynthesis occurring under insufficient light conditions. The height of the tomato was significantly increased when planted without blue light or low blue light conditions (Naznin & Lefsrud, 2014). Therefore, the short stem under LED and fluorescent light might promote a higher composition of blue light intensity and ratio than in the greenhouse rendering of inhibition of stem elongation. Meanwhile, LED light from 200 µmol/m²/s also resulted in better growth and development of stem, root and leaf parts, as well as chlorophyll content of tomato seedlings. This development resulted in an increased health index of tomato seedlings which higher than tomato seedlings grown in greenhouses. While the tomato seedling exposed to LED light at equal to or less than 100 µmol/m²/s and fluorescent light resulted in

similar growth and developed in that tomato seedlings grow under greenhouses (Fig 4.1). Fan et al., 2013 found that fresh weight, dry weight, and health index of young tomato seedlings were significantly increased when grown under red and blue LEDs at equal to or more than 300 µmol/m²/s. The light intensity at 300 µmol/m²/s was shown to be suitable for growing young tomatoes, while a light intensity greater than 300 µmol/m²/s resulted in a decrease in photosynthetic efficiency (Fan et al., 2013). Similar with Yao et al., 2017 reported that LED light less than 400 µmol/m²/s led to the accumulation of biomass and photosynthesis products. They concluded that the light intensity at 400 µmol/m²/s is suitable for *Brassica napus* L. growing. While the light at 389 µmol/m²/s has the best effect on the yield of Batavia red lettuce cv 'Blackhawk' (Modarelli et al., 2022). The low intensity may result in a limitation in photosynthesis. In contrast, the high light intensity can induce plant stress and lead to inhibition of the plant's photosynthetic (T. Lu et al., 2019; Ma et al., 2010). From the results, when considering the health index of seedlings to assess the suitable light for seedling growth, it was found that an LED at 200 µmol/m²/s was suitable for the tomato and chili seedlings planting. The LED light at 300 µmol/m²/s was suitable for the melon and mustard green seedlings and the LED light at 400 µmol/m²/s was suitable for the growth of chinese kale seedlings. The results demonstrated the influence of specific light intensity on seedling growth in each plant species. Those light intensities may be sufficient and suitable for the growth and development of those seedlings and resulting in a high health index seedling. While over light intensity, the plant may be adapted by reducing the exposure by reducing the leaf area (Milthorpe & Newton, 1963; Modarelli et al., 2022).

In terms of the effect of the optimum light ratio on the seedling growth when planted under the appropriate light intensity, it was found that the light ratio at R80:B20 resulted in the highest plant height of tomato seedlings when compared with other treatments (Fig 4.4). The red and blue light played a role in regulating plant growth and development, for example, red light had a negative effect on carbon dioxide uptake, electron transport in photosynthesis, and the accumulation of plant biomass. However, the red light promotes hormone production, stem elongation, leaves, and root development (Alallaq et al., 2020; Hoenecke at al., 1992; Yan Li et al., 2020; H. E. Oh et al., 2021; OuYang et al., 2015; Spaninks, van Lieshout, van leperen, & Offringa, 2020). While blue light had a negative effect on stem elongation but has a positive effect on stimulating stomata opening, phenolic compound formation, carbon dioxide absorption, and photosynthesis (Inoue & Kinoshita, 2017; Suetsugu et al., 2014; Toh et al., 2018; Zeiger & Hepler, 1977; Zheng & Van Labeke, 2017). OuYang et al., 2015 reported that high gibberellin production induced by red light leads to stem elongation. Naznin & Lefsrud, 2014 found that the height of tomato plants significantly increased when exposed to red light alone or red combined with blue in a ratio of 10:1, but the rate of photosynthesis was greatly reduced when grown under a single red light. In addition Yorio et al., 2001 reported that lettuce, spinach, and radish were planted under a high level of red light caused a negative effect on biomass accumulation. Therefore, the highest plant height was found under LED light at the ratio of R80:B20, possibly due to high red light exposured which might lead to the longest stems. At the same time, the root length, root fresh weight, root dry weight, root/shoot ratio, and leaf area were reduced when planted under LED light at R40:B60 and R20:B80 when compared with R50:B50, R60:B40, and R80:B20. While the chlorophyll content was the highest under the light ratio at R50:B50 (Fig 4.4). Meng et al., 2019 found that planting Gerbera jamesonii under red and blue or red and blue LEDs at 50%:50% resulted in a significantly reduced root length. While root was the longest when planted under red and blue at 60%:40%, which red light promotes root development by inhibiting the production of phytohormones such as jasmonate (JA), cytokinins (CKs) (Alallag et al., 2020). In addition, both *phyA* and *phyB* were play a role in photo-stimulated root elongation (Correll & Kiss, 2005). Wu, 2012 reported that red LED light promoted root development of Protea cynaroides L. better than blue light alone or red light combined with blue and fluorescent light. J.-H. Yang et al., 2015 found that the red light resulted in longer leaves. The increase of roots and the leaf area due to the high red light ratio the plants received, may be related to the hormonal system and has the effect of promoting the development of roots and leaves of tomato seedlings. In addition, tomato seedlings grown under the ratio at R50:B50 and R80:B20 had higher fresh weight, dry weight, and stem diameter than seedlings planted at R40:B60 and R20:B80 (Fig 4.4). These phenomena were most directly related to the health index seedling. When considering the health index of seedlings, it was found that a light ratio at R60:B40 resulted in the highest health index of tomato, melon and chinese kale seedlings. While the light ratio at R50:B50 resulted in the highest health index of chili and mustrard seedlings (Fig 4.6) Meng et al., 2019 reported that the light ratio at R70:B30 or R60:B40 results in *Gerbera jamesonii* efficient photosynthesis and they concluded that this ratio of light was suitable for growth. Similarly, Yan Li et al., 2020 demonstrated the combination of red light with blue at a ratio of R75%:B25% or blue light alone promotes the genes involved in photosynthesis and enzymatic activity related to the Cavin cycle in sweet pepper better than red light alone. Therefore, the results concluded that the combination of red light with blue at a nearby ratio of R60:B40 or R50:B50 may be a suitable ratio for the photosynthesis of these seedlings. Whereas exposure to more of a particular wavelength alone may have a negative effect on the photosynthesis of seedlings. Thus, cause the amount of chlorophyll and biomass accumulation were reduced and led to a decrease in the health index seedling.

In terms of light photoperiod on seedling growth, it was found that the illumination of fluorescent lighting for 10/D, the plant height of tomato seedlings was similar to a greenhouse. But when it was exposed to the fluorescent light at 12 h/D and LED light at 10 h/D ascend, the height was significantly reduced when compared to the greenhouse. While the increasing the photoperiod at 10, 12 and 14 h/D resulted in a sequentially increase in plant, root, and leaf development. Those parameters showed the highest when tomato planted under appropriate light intensity, light ratio and provide light for 14 h/D, (Fig 4.7). Also, when using appropriate LED light and providing illumination for 14 h/D, the health index of tomato, melon, chili, and mustard green seedlings demonstrated the highest (Fig 4.9). While the chinese kale seedlings had the highest health index when provided light for 12 h/D (Fig 4.9). Consequently, the tomato and melon, chili, and mustard green seedlings were investigated with a light photoperiod longer than 14 h/D. It was found that tomato seedling was significantly decreased in plant height when fluorescent light illuminated at 20 h/D and LED light at 14 h/D ascend. While root length and leaf area were significantly increased when planted under fluorescent and LED light from 14 h/D up. The chlorophyll content and biomass were significantly increased in tomato seedling when fluorescent light illuminated equal to or more at 18 h/D

and LED light equal to or more at 14 h/D (Fig 4.10). When considering the health index of tomato seedlings, it was found that LED light illuminated at 24 h/D resulted in the highest health index but was not significantly different when compared to LED light was illuminated at 20 h/D. The health index of melon seedlings was the highest when LED light illuminated at 20 h/D and decreased slightly when LED light was exposed at 24 h/D. The highest health index of chili seedlings was found under LED light illuminated at 24 h/D, however, was not significantly different from the LED light illuminated at 18 h/D. The mustard green seedling was highest health index when plant under the LED light illuminated at 18 h/D and was significantly reduced when grown under LED light illuminated at 20 and 24 h/D (Fig 4.11). Many plant processes were affected by the response of the gene network to changes in the photoperiod cycle (Osnato et al., 2022). The increase in chlorophyll content under long photoperiod enhances light absorption, chemical reaction changes and promotes the growth of Rudbeckia fulgida var. sullivantii 'Goldsturm' seedlings (Elkins & van Iersel, 2020). Huang et al., 2021 reported that the plants are exposed to light beyond the saturation point, leading to a decrease in biomass accumulation. The growth, chlorophyll content and carbohydrate accumulation of Sulhyang and Maehyang were reduced when planted with exposure greater than 20 h/D (Yali Li et al., 2021). The results obviously showed that short periods resulted in poor plant growth, development, and low unhealthy. However, the over photoperiod does not improve plant development and growth more or even has a negative effect on plant growth as well. The seedlings were grown under optimal light intensity and light ratios from LED light for each seedling include tomato (200 µmol/m²/s, R60:B40), melon (300 µmol/m²/s, R60:B40), chili (200 µmol/m²/s, R50:B50), mustard green (300 µmol/m²/s, R50:B50), and chinese kale (400 µmol/m²/s, R60:B40), and illuminated at 20h/D for tomato and melon seedlings, at 18 h/D for chili seedlings and mustard green and at 12 h/D for chinese kale seedlings were the optimum light condition for growth (Table 4.1). This might be the saturation point of the seedling's ability to light. Since the longer exposure, the parameters did not increase or even decrease as well. This might be the result of the plant receiving light over the saturation point of using light and lead to a negative effect on seedlings which is not suitable for growth.

The effects of PGPR on seedling growth, the result found that bacterial isolates SUTN9-2, A20, and SD10 significantly promoted plant height, shoot fresh weight, root fresh weight, roots dry weight, stem diameter, and leaf area of tomato seedlings (Fig 4.13). While other treatments showed negative effects on these parameters. While SD10 had a significantly increased in chlorophyll content, but 3D13 had a significantly reduced chlorophyll content when compared to control (Fig 4.13). When considering the health index of seedlings, it was found that the tomato seedling inoculated SUTN9-2 showed the highest health index, followed by SD10. However, SUT19, S141, Ch12, 3D13 and Cat697 had a negative effect on the growth and health of tomato seedlings. While the melon seedling inoculated with SUTN9-2 resulted in the highest health index. In the chili seedlings inoculated with SUT19 performed the highest health index, followed by SUTN9-2. For the mustard green seedlings, the inoculated SD10 was highest health index, followed by the inoculated with SUT19. In the case of chinese kale seedlings, the inoculated with S141 showed the highest health index, followed by inoculated SUT19 (Fig 4.15). According the PGPR strains used in the experiment display several important properties for promoting plant growth, such as ACC deaminase, IAA synthesis, nitrogen fixation, phosphate dissolving, and as a biological regulator (Table 4.2). Gupta & Pandey, 2019 reported that Aneurinibacillus aneurinilyticus and Paenibacillus sp produced the IAA and phosphate solubility, production of siderophores, and ammonia could promote the French bean seedling growth. However, some strains of bacteria are good at promote shoot growth, while some species good promote the root development (Lwin et al., 2012). Changes in the plant root might be result from interference of PGPR with key hormonal pathways involved in the regulation of plant root development, such as auxin, cytokinins, ethylene, and amplified gibberellins, and abscisic acid (ABA), most of PGPR regulates the root system by regulating hormonal balance (Vacheron et al., 2013). Many reports showed PGPR produced auxin affect root development (Etesami & Alikhani, 2016; García et al., 2002). Previous reports showed that PGPR could promote the Brassica oleracea seedlings growth (Turan et al., 2014), possibly reasons for the increasing of shoot development and health index may be related to various mechanisms of PGPR co-inoculated with seedlings. PGPR might be enhanced the absorption of nutrients needed for photosynthesis and protein synthesis of plant seedling. While the increasing root development may due to influenced by alterations in auxin levels. However, excessive amounts of auxin could promote harmful effects on plants such as reduce root growth (D. Duca et al., 2014).

The results showed that the tomato seedlings inoculated with SUTN9-2 and SD10 and planted under light conditions had no effect on the plant height, root length, and leaf area (Fig 4.16). This might be attributed to the light conditions having a more severe effect on the control of stem and root elongation, and leaf expansion than the effect of PGPR. However, the tomato seedlings inoculated SD10 and planted under greenhouse condition showed the significantly increased biomass and stem diameter when compared to the control. While the LED illuminated tomato seedling and inoculated SD10 was significantly increased in root fresh weight, root dry weight, and total dry weight when compared to LED illuminated tomato seedling without inoculate. The fluorescent illuminated tomato seedling inoculated with SD10 resulted in root dry weight, stem diameter, and the root/shoot ratio significantly increased. When considering the tomato health index, it was found that the tomato seedlings inoculated with PGPR and planted under a greenhouse and the LED light had no significantly effect on the health index (Fig 4.16). While the tomato seedling health index was significantly increased when inoculated with SUTN9-2 and illuminated under fluorescent light. However, the LED illuminated tomato seedling showed higher in health index than other light conditions. Moreover, the LED illuminated tomato seedling and inoculated SD10 showed the highest health index. In addition, the LED illuminated melon and pepper seedlings and inoculated SUTN9-2 resulted in highest health index. For the chinese kale seedlings, the highest health index showed when LED illuminated chinese kale seedlings inoculated whit S141. The LED illuminated mustard green seedlings showed highest health index (Fig 4.18). The results investigated that, the co-inoculation of PGPR with seedlings under different light conditions showed the different of seedlings growth. Possibly reasons for the seedling growth increasing have already been discussed in the previous paragraph. Roots are the most important organs of plants which serves to absorb nutrients and water effectively. At the same time, during plant grows, certain substances are released through the roots called root exudate (Matusova et al., 2005). Root exudates affect the microbial population surrounding the roots (Nazir et al., 2016; Williams & de Vries, 2020) Previous reports suggested that light quality and intensity affect the quantity and component of root exudates (L. Yang, 2016; Zhou et al., 2020), Therefore, the transformation of root exudates of plants under different light conditions may influence on the variant PGPR metabolites, which might be the reason that the co-inoculation of PGPR with seedlings under different light conditions affects the growth of different tomato seedlings.

The AMF inoculation with seedlings was produced under different conditions. The results showed that the tomato seedling inoculated with SD10, AMF, and SD10 combined with AMF were able to promote the growth in both seedlings produced under greenhouse and LED light after transplant to the greenhouse at 10 and 30 days. The LED illuminated tomato seedling inoculated with SD10 and co-inoculated AMF showed the greatest biomass accumulation when compared to other treatments after transplant to the greenhouse at 30 days. However, the root colonization was lower when AMF was inoculated with tomato seedlings inoculated with SD10. This reduction in root colonization might be attributed to the influence of SD10. Since, the SD10 may have a mechanism to inhibit AMF growth and AMF infestation in tomato roots, because the one of characteristics of SD10 is biocontrol (Fig 4.19 and 4.20). Pérez-de-Luque et al., 2017 reported that the number of AMF/unit/root lengths was significantly reduced when Pseudomonas putida KT2440 was inoculated with *Rhizophagus irregularis* in Mercato plants. For the melon, it was found the LED illuminated melon seedling and inoculated with SUTN9-2 and SUTN9-2+AMF resulted in a slightly reduced stem diameter. Whereas inoculated with AMF alone, the diameter was slightly increased after transplant to the greenhouse at 10 days when compared to LED illuminated melon seedling. When comparing greenhouse melon seedlings, the LED illuminated melon seedlings resulted in fresh weight and biomass were increased. Moreover, the LED illuminated melon seedling and inoculated with SUTN9-2 resulted in the highest biomass when compared to the other treatments, while the root colonization was not significantly different (Fig 4.22 and 4.23). In the chili seedlings, it was found that the LED illuminated chili seedling and inoculated or non-inoculated with PGPR showed higher stem diameter and chlorophyll content than chili seedlings produced in a greenhouse after transplant to greenhouse at 10 days. Then after transplant to the greenhouse at 30 days, the LED illuminated chili seedling and inoculated or non-inoculated with PGPR showed resulted in a significantly increased in shoot and root growth when compared to seedlings produced in a greenhouse. However, the LED illuminated chili seedling and inoculated with SUTN9-2 showed the highest biomass accumulation (Fig 4.25 and 4.26). Significantly increased growth of LED illuminated chili seedlings in both inoculation and

non-inoculation of PGPR at 30 days after transplant in a greenhouse. This might be attributed to during 10 days after transplant, the chili seedlings had high chlorophyll content may high photosynthesis activity and carbohydrate accumulation then can grow quickly in the next phase. Subsequently, the chlorophyll content was the same in all treatments after 30 days of transplanting in the greenhouse. This might be caused by chili seedlings adapting the amount of chlorophyll according to the light environment in the greenhouse. The increase of biomass or changes in various development of the seedling may be attributed to the activity of PGPR and AMF the properties associated with the their metabolites such as nitrogen fixation, ACC deaminase production, plant hormone production, phosphate dissolving, and adsorption enhancement of water and various nutrients for plants. M. Carrillo et al., 2014 reported that *Rhizophagus intraradices* promotes plant height and chlorophyll content of tomato plants. Begum et al., 2022 reported that inoculation of PGPR and AMF alone or in combination promoted growth and photosynthesis of tobacco by regulating various metabolites. L. Yu et al., 2022 reported that co-inoculation of Bacillus megaterium with Funneliformis mosseae was effective in promoting the biomass accumulation and development of the shoot and root of the *Elymus nutans* Griseb.

The fruit yield in the field experiment of tomato seedlings at 30 days after the start of harvesting, it was found that the tomato seedlings produced under suitable LED light and inoculated SD10+AMF resulted in a 16% increase in fruit weight (Fig 4.28). This increase in yield might be attributed to a 7.98% increase in fruit number when compared to the yield of tomato seedlings produced in the greenhouse. Son et al., 2018 reported that the red and blue LED light ratios to have a direct effect on the growth of cherry tomato seedlings and may affect reproductive growth. The AMF inoculation with *Prunella vulgaris* has been reported to result in increased flowering (T. Young et al., 2015). While inoculation of *Glomus mosseae* accelerates flowering

and fruit development and increases tomato yield (A. Salvioli et al., 2012). Inoculation of rhizobium, PGPR and AMF, alone or in combination, can promote *Vicia faba* L. and *Triticum durum* L. yields (Raklami et al., 2019).

Yield in the field experiment of chinese kale was recorded at 50 days, it was found that the LED illuminated chinese kale seedlings inoculated with S141 resulted in 13.82% increase in yield when compared to chinese kale seedlings produced in greenhouses (Fig 4.29). The increase in yield may be attributed to LED illuminated chinese kale seedlings and inoculated S141 had a high health index. Then, when transplanted to the field there may be an adaptation to the environmental conditions and grow faster than chinese kale seedlings produced in greenhouses. Production of lettuce seedlings under blue and blue combined with red light resulted in lettuce plants growing better after transplant in the greenhouse (Johkan et al., 2010). The inoculation *Bacillus mycoides* T8 and *B. subtilis* OSU-142 alone or in combination had the potential to promote the yield and vegetative growth of the Sour cherry (Arikan & Pirlak, 2016). Therefore, healthy seedling production by using LED light in combination with PGPR and AMF presented the potential to increase productivity in the experimental field.

The high activity of SOD might be a mechanism responding to the light stress of tomato from the LED light condition, then it induced a high accumulation of H_2O_2 in tomato laves refer to as brown leave (Fig4.30a and b). Blue light induced ROS production and SOD activity (Lee et al., 2014; Rossa et al., 2002). The *psbB* gene is localization on plastid genome, it encoded CP47 protein CP47 protein is pigment-binding protein was found on PII complex protein. The main function of CP47 is inner light harvesting complex and drive it in form of excitation energy to photochemical reaction proteins (D1,D2 proteins) (Luci**ń**ski & Jackowski, 2006; Weerd, Stokkum, Amerongen, Dekker, & Grondelle, 2002). Kim et al., 2014 reported that the shorter wavelength green and blue LED lights induced the expression of *pebA* and *psbB* genes in *Synechococcus* sp.. The *psbA* gene encoded D1 protein, it is one of core protein on PSII reaction center, act as radiation energy transformation through oxidation of water and reduction of plastoquinone (Singh, 2000). Although light is necessary for photosynthesis, whereas the excessive or unsuitable of light conditions lead to PSII damage referred to photoinhibition. Photoinhibition occurs when the rate

of damage exceeds the rate of PSII repair, D₁ protein is highly sensitive to photodamage (N. Ahmad et al., 2020; Kale et al., 2017; Takahashi & Badger, 2011; H. Wu et al., 2011). Our result found that the expression level of *psbA* and *psbB* genes shown significantly upregulated on tomatoes seedling grown under LED light condition when compared to tomatoes seedling grown under greenhouse condition. The light quality could control the rate of transcription level on chloroplast genome as *psbA* and *psbB* (T. Pfannschmidt et al., 1999). The mixtures of red and blue light as low light intensity (50, 100 μ mol/m²/s) could increases the gene related with photosynthetic including *Lhcb4.2*, *Lhcb6*, *psbA*, *psbB*, and *psbD* genes, the increasing of those genes might be the strategy to protect the photosynthetic machinery and enhance the potential of photosynthetic system (X. Wu et al., 2021). Therefore, light-induced the psbA mRNA might be triggered by D1 damage, while the increase in translation elongation rate may be triggered by a product of photosynthesis (Chotewutmontri & Barkan, 2020).

The *FDX* gene encoded for the ferredoxin proteins production. The ferredoxins function by accept the electron from PSI and then it transferred to the flavo-enzyme ferredoxin:NADP(H) oxidoreductase (FNR) to NADPH synthesis. The ferredoxins play important roles in electron transport chain in photosynthesis, CO_2 assimilation, nitrate, sulfate, and other metabolites (Chen et al., 2021; Kozuleva et al., 2016; Liu et al., 2019). Ferredoxin is the major iron-containing protein found in the photosynthetic organisms and central to reductive metabolism in the chloroplast (Terauchi et al., 2009). The previous study reported that the light could regulated the expression of FDX gene in plants such as Arabidopsis thaliana., Nicotiana tabacum L. and Pisum sativum L. (Bovy et al., 1995; M. Gallo-Meagher et al., 1992). This study found that upreglulation of FDX gene in the tomato seedling planted under LED light condition when compared to tomato seedling planted under greenhouse condition. The FDX showed the highest expression in *Codonopsis lanceolata* seedling when treated under composition of red and blue light. The abundant of FDX expression may be regulated by the blue and red lights (Ren et al., 2018). While the overexpression of ferredoxins gene, including PETF and FDX5 in Chlamydomonas reinhardtii could enhance heat tolerance, salts stress, also induced starch and oil accumulation, and raise electric power density in a photo microbial fuel cells (Huang et al., 2015). Therefore, up-regulated of *FDX* gene may induced by LED light condition, which higher expression of *FDX* may enhanced NADPH synthesis in photosynthetic system. The high level of NADPH may lead to photosynthesis, resulting in high biomass. The *atpB* gene-encoded chloroplastencoded β -subunit of ATP synthase. ATPases are the enzyme that catalyze the adenosine triphosphate (ATP) form adenosine diphosphate (ADP), driven by the electrochemical proton gradient created by light-dependent photosynthetic electron transport (Strotmann & Schumann, 1983). However, the oxidative stress caused the degradation of RbcL and atpB proteins (J. Li et al., 2022). The *atpB* and expression may response in light quality (Valle et al., 2014; H. Zhang et al., 2019). Therefore, up-regulated of *FDX* and *atpB* gene may induced by LED light condition, which higher expression of *FDX* and *atpB* may enhanced NADPH and ATP synthesis in photosynthetic system.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is an enzyme catalyzed in the photosynthetic system and acts as CO₂ fixation in the first of the Calvin cycle. It is located in chloroplasts of higher plants and encoding by *rbcL* and *rbcS* genes, large and small subunits, respectively (Manzara & Gruissem, 1988; Portis & Parry, 2007; Yamada et al., 2019). The increase of *rbcL* and *rbcS* mRNA could be induced by light, also the abundance of *rbcL* and *rbcS* proteins were found when peas were illuminated by light (Inamine et al., 1985). The Ribulose-1,5-bisphosphate carboxylase small subunits mRNA and enzyme were increased by illumination (Sasaki et al., 1981). Y. Li et al., 2020 reported that the higher activity of RuBisCo was found when chili seedling treated with blue or combination of red and blue light, meanwhile the combination of red and blue light resulting in highest biomass. Similarity, the Codonopsis lanceolata seedlings was exposed under mixture of red and blue light resulting in significantly up-regulation of *rbcL*. This study suggested that the significantly up-regulation of *rbcL* was found with tomato seedling treated under LED light condition when compared to greenhouse condition. While the rbcS expression showed slightly increase when treated under LED. The results revealed that under the optimum LED light may influence on the rbcL and rbcS genes expression and high level of rbcL and rbcS mRNA may led to the carbohydrate synthesis and biomass accumulation in tomato seedling.

CHAPTER VI DISCUSSION

Light plays a major role in the regulation of plant growth and development. The application of LED light in terms of quality and quantity could promote the plant seedling for high quality. However, different plant species require light in different conditions. The LED light involved in the regulation of the photosynthetic genes expression. While some strains of PGPR such as *Bradyrhizobium* sp. SUTN9-2, *Pseudomonas* sp. SUT19, *Bacillus velezensis* S141, and *Bacillus velezensis* SD10 could promote seedling growth in the greenhouse, or even when used in PGPR inoculated with LED illuminated seedlings in the case of SUTN9-2, S141, and SD10. However, some strains showed a negative effect on seedling growth. Also, the PGPR inoculated with LED illuminated seedlings in combination AMF could promoted seedling growth in transplanting state. Finally, the high quality seedling was produced by LED light and beneficial microorganisms could promoted yield production.

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Figure A.1 The effect of light intensity on melon seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$



Figure A.2 The phenotype of melon seedling growth under different light intensity. Wash planting material (a), non-wash planting material (b).





Figure A.3 The effect of light intensity on chili seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.4 The phenotype of chili seedling growth under different light intensity. Wash planting material (a), non-wash planting material (b).







Figure A.5 The effect of light intensity on mustard green seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.6 The phenotype of mustard green seedling growth under different light intensity. Wash planting material (a), non-wash planting material (b).









Figure A.7 The effect of light intensity on chinese kale seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.8 The phenotype of chinese kale seedling growth under different light intensity. Wash planting material (a), non-wash planting material (b).







Figure A.9 The effect of light ratio on melon seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.10 The phenotype of melon seedling growth under different light ratio. Wash planting material (a), non-wash planting material (b).







Figure A11 The effect of light ratio on chili seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.12 The phenotype of chili seedling growth under different light ratio. Wash planting material (a), non-wash planting material (b).









Figure A.13 The effect of light ratio on mustard green seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.







Figure A.14 The phenotype of mustard green seedling growth under different light ratio. Wash planting material (a), non-wash planting material (b).










Figure A.15 The effect of light ratio on chinese kale seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.16 The phenotype of chinese kale seedling growth under different light ratio. Wash planting material (a), non-wash planting material (b).





Figure A.17 The effect of light photoperiod on melon seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.18 The phenotype of melon seedling growth under different light photoperiod. Wash planting material (a), non-wash planting material (b).





Figure A.19 The effect of light photoperiod on chili seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.20 The phenotype of chili seedling growth under different light photoperiod. Wash planting material (a), non-wash planting material (b).



Photoperiod

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Figure A.21 The effect of light photoperiod on mustard green seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.22 The phenotype of mustard green seedling growth under different light photoperiod. Wash planting material (a), non-wash planting material (b).









Figure A.23 The effect of light photoperiod on chinese kale seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.24 The phenotype of chinese kale seedling growth under different light photoperiod. Wash planting material (a), non-wash planting material (b).









FigureA.25 The effect of light photoperiod at 14–24 h/D on melon seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.26 The phenotype of melon seedling growth under different light photoperiod at 14–24 h/D. Wash planting material (a), non-wash planting material (b).







Figure A.27 The effect of light photoperiod at 14–24 h/D on chili seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.28 The phenotype of chili seedling growth under different light photoperiod at 14–24 h/D. Wash planting material (a), non-wash planting material (b).





Photoperiod





Figure A.29 The effect of light photoperiod at 14–24 h/D on mustard green seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.30 The phenotype of mustard green seedling growth under different light photoperiod at 14–24 h/D. Wash planting material (a), non-wash planting material (b).









Figure A.31 The effect of PGPR on melon seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.32 The phenotype of melon seedling when co-inoculated with PGPR. Wash planting material (a), non-wash planting material (b).







Figure A.33 The effect of PGPR on chili seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.34 The phenotype of chili seedling when co-inoculated with PGPR. Wash planting material (a), non-wash planting material (b).







Figure A.35 The effect of PGPR on mustard green seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.36 The phenotype of mustard green seedling when co-inoculated with PGPR. Wash planting material (a), non-wash planting material (b).







Figure A.37 The effect of PGPR on chinese kale seedling growth. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.38 The phenotype of chinese kale seedling when co-inoculated with PGPR. Wash planting material (a), non-wash planting material (b).

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Figure A.39 The phenotype of melon seedling when inoculation with PGPR and planted under optimum light condition. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.40 The phenotype of melon seedling when inoculation with PGPR and planted under optimum light condition. Wash planting material (a), nonwash planting material (b).



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Figure A.41 The phenotype of chili seedling when inoculation with PGPR and planted under optimum light condition. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.42 The phenotype of chili seedling when inoculation with PGPR and planted under optimum light condition. Wash planting material (a), non-wash planting material (b).



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Figure A.43 The phenotype of mustard green seedling when inoculation with PGPR and planted under optimum light condition. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with differen letters in each treatment are significant different at $P \le 0.05$.



Figure A.44 The phenotype of mustard green seedling when inoculation with PGPR and planted under optimum light condition. Wash planting material (a), non-wash planting material (b).





Figure A.45 The phenotype of chinese kale seedling when inoculation with PGPR and planted under optimum light condition. Shoot height (a), root length (b), shoot fresh weight (c), root fresh weight (d), shoot dry weight (e), root dry weight (f), total fresh weight (g), total dry weight (h), stem diameter (i), leaf area (j), chlorophyll content (k), root/shoot ratio (l), Mean and standard deviation are calculated from four replicates, and values with different letters in each treatment are significant different at $P \le 0.05$.



Figure A.46 The phenotype of chinese kale seedling when inoculation with PGPR and planted under optimum light condition. Wash planting material (a), non-wash planting material (b).

BIOGRAPHY

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