INVESTIGATING LED LIGHTING SPECTRA TO IMPROVE PLANT GROWTH AND ENERGY USE EFFICIENCY

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ABSTRACT

Investigating LED Lighting Spectra to Improve Plant Growth and Energy Use Efficiency

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The climate change, natural resource collapsing, the world population increase, and accompanied by the urbanization movements, are forging the global food security issue. Vertical farming as an agriculture mode in the urban areas is designed to meet the city food requirement as a priority, at the same time, the emergence of LED semiconductor light source not only provides an ideal controllable light source for plant cultivation, also offers a mythology for plant photobiology study. It qualified the LED light to be the option meeting the overall requirements for modern agricultural production mode, such as high efficiency, high yield, top quality, ecology and safety, which consequently engaged this lighting method with extensive application prospects.

The research was mainly conducted with pak choi (*Brassica rapa, Chinensis*), to detect the difference physiological response and gene expression under treatments of the changed LED spectra. LED lighting can benefit the spectral output by becoming adjustable and species-specific for generating the appropriate 'light recipes' covering the plant consistently changing requirements, according to the growth stages, developing appeals and the

species differences. This study exploits new developments in a low cost, efficiency, and new LED lighting system with the optimised set of wavelengths which needed to maximise yield with suitable trade-offs on energy input. In pak choi growth, the red/blue ratio as 70%/30% LED treatments showed positive effects on pak choi, as the yield can be increased by 50% compared with fluorescent lamp. Also, the LED lights showed remarkable improvement in energy saving and flexibility during production. In addition, the blue light shows significant affection for plant photomorphogenesis and light stress tolerance in mixed and monochromatic light. The CO_2 absorption was also strongly regulated by the proportion of blue light, which indicate the fact that the ratio of red and blue light was the deciding factor in balancing light reaction and the Calvin cycle. The chlorophyll fluorescence measurements suggested 73% red / 27% blue ratio LED light recipe can increase the efficiency in PSII centre and carbon fixation which related to fresh matter increasing. Furthermore, a large number of differential expressed genes was detected by RNA-sequencing in pak choi，which showed genome-scale insight of gene expression responded under different light treatments.

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ABBREVIATION

ANOVA	Analysis of variance
Chl	Chlorophyll
Ci	Intercellular CO_2 concentration
CO_2	Carbon dioxide
Cond	Stomatal conductance
DEG	Differential expressed gene
ELIP	Early light-induced proteins
Fv/Fm	Optimal phorochemical efficiency of PS II
LED	Light Emitting Diode
PAR	Photosynthetically active radiation
PhiPS2	Quantum yield of photosystem II
PIF	Phytochrome Interacting Factor
Pn	Net photosynthetic rate
PPFD	Photosynthetic Photon Flux Density
PSI	Photosystem I
PSII	Photosystem II
qP	Photochemical quenching
SAS	Shade-Avoidance Syndrome
SPAD	Chlorophyll content measured by SPAD

Chapter 1

INTRODUCTION

Chapter 1: Introduction

1. 1 Food Security: The effects of global population and urbanization

Since the 21st century, the increasing world population is one of the major concerns when it comes to the global food security issue. The gap between the restricted food-producing capacity and the world growing food demand is the primary impedances of human beings' future survival. Based on the present database, the global population is anticipated to reach 9.8 billion in 2050 (WRI, 2018), the world will need to increase food production by more than 50 % to feed nearly 10 billion people adequately in 2050 (Tim S. et al., 2018).

On the other hand, urbanization is a trend unique to the past few centuries. Projected by United Nation that by 2050, more than two-thirds of the world population will live in urban areas, which indicated that close to 7 billion people will live in cities (Will V. and Hannah W. 2019). This change leads to several consequences to our traditional agriculture: (1) Large numbers of people is moving to cities, resulting in the loss of agricultural labor; (2) Economic, technology and other resources will be concentrated in large cities; (3) Agriculture directly serves the urban residents, who require food to be more various, personalized, and high-quality products. All these trends bring us new challenges, consequently, lead revolutionary changes to the structure of production chain, crop growth management module, and innovative technology

(FAO, 2016).

1. 2 Vertical farming and artificial lights

In the conventional sense, the urbanization brought a negative impact on agriculture industries as this phenomenon means eliminated farming workforce and resource contention. Contrastingly, scientists realize that urbanization is more likely to be an opportunity. Thanks to technology improvement, urban farming as a leading-edge concept, now is considered as a possible solution to acquiring farmable sites for modern agriculture (Suparwoko and Betri T. 2016). The concept of vertical farming was brought by Dickson Despommier, the professor in Environmental Health Science department of Columbia University and his students, who stated that vertical farming is recognized as a promising area as it uses underutilized space of city buildings (roof top, basement, abandoned factory or stations) with advanced greenhouse technology such as artificial lights, hydroponics/aeroponics and environment control methods, to produce vegetables, fruits, and other crops year-round (Dickson D. 2010).

On the other hand, this in-door form of vertical farming or urban farming indicated that we must supply all the conditions for crop growth by artificial measures. Light, as the most important growth factor which drives photosynthesis and strongly affects plant growth, is considered to be the most critical condition in this closed environment. The application of artificial light sources in cultivated plants has been more than 150 years history and has gone through four stages: incandescent lamps, open arc lighting, enclosed gas

discharge lighting and Light-emitting diode (LED) solid-state lighting (Lin Y. et al., 2011). At present, the field of plant supplementation mainly uses fluorescent lamps as artificial light sources, but LED is now considered as the most prospective advancement in the following decades, this light source devices with its advantages such as high efficiency, narrow spectral, environmental friendly, cold working temperature, and long lifetime, has now begun to enter the stage of rapid development, and appeared in a large number of urban agricultural applications (Morrow R. C. 2008).

1. 3 Problem statement and Justification

Although, as a resource-intensive mode of agricultural production, urban agriculture and vertical farming can alleviate food crisis caused by population increasing and urbanization, we still need to maximize the efficiency of resource utilization due to its high costs. Photosynthesis provides all the energy for plant growth which makes it the key progress for efficiency improving, and the photosynthetic pigments are involved in light energy absorption, transmission and conversion, their content and composition directly affect photosynthetic efficiency. As the most critical pigment involved in photosynthesis, chlorophyll's absorption peaks are mainly dropped in 430nm-470nm and 650nm-675nm, namely red and blue light. Therefore, the “Light Recipe” dominated by red and blue light has become an important way for researchers to study crop photosynthesis (Savvides A. et al., 2012; Zeiger E. et al., 1981). With the development of LED technology and the decreasing of manufacturing cost, the application of LED light source (especially the combination of red and blue LED lights) in urban agriculture has attracted more and more attentions (Singh S. et al., 2017), such as Kim S. J. et al (2004) indicated that mixed red and blue light

was more conducive to the growth of lettuce, and higher light intensity led to higher photosynthetic rate; also Lin Y. et al (2011) found that the concentrations of chlorophyll and carotenoids in the protocorm-like bodies of Dendrobium officinale could be significantly increased if treated by different red-blue ratio LED lights.

However, the adoption of LED lights in vertical farming is still limited by the plant photobiology speciality and the characteristics of LED devices themselves, to be specific: (1) The vegetative and reproductive growth of agricultural crops are diverse and complex; (2) The number of crops varieties involved in agricultural production is extremely huge. (3) Light conditions involve many properties such as lighting spectrum, intensity, period, heat dissipating, and all these properties need to be investigated and matched to crops growth conditions (Liu W. et al., 2012). The combination of these three reasons has greatly increased the complexity of LED photobiology research, as LED lights are still cutting-edge and evolving technology, it is urgent for us to target a particular crop, focus on the effects on plant growth and the photobiological mechanism behind it (Yang Y. T. et al., 2010).

The crops from brassica family have attracted a lot of attention as they are rich in beneficial phytochemicals (Anderson M. et al., 1985), such as broccoli (*Brassica oleracea var. italics*), cabbage mustard (B. Oleracea var. ace-gala) and pak choi (*B. rapa ssp. chinensis*) have been confirmed as rich source of vitamin C, carotenoids, and minerals (Hanson P. et al., 2009). In Asia, pak choi is one of the most widely eaten vegetables due to its rapid growth, convenient management, and lower price. The technology of Brassica family cultivation in facilities agriculture has been quite mature, but its growth under artificial light, especially LED lights, has not been studied much. Based on the

idea of expanding research scope and enriching plant varieties, we chose pak choi as representatives of the Brassica family to carry out a series of research in order to gain a deeper understanding of these wide varieties.

To further explore the regulation of genes on plant growth, transcriptome has been developed to investigate the transcription and regulation at molecular level. RNA-seq (RNA Sequencing), also known as Transcriptional Sequencing technology, is using high-throughput sequencing technology to determine the sequence of mRNA, small RNA, and Non-coding RNA, for in-depth study of transcriptional complexity (Luca V. et al., 2013). In order to explore the mechanism of how light conditions impact plant growth, the experiment is to adopt transcriptomic sequencing analysis detecting the target genes of which impacting on the object plants' growth across different LED light recipes and plant varieties.

1. 4 Objectives

In general, the LED light is currently a good choice as a commercial artificial light source, with its prime advantage of photoelectric conversion efficiency and the high applicability in the agricultural field, yet there are not enough accurate or reliable study for a wide range of agricultural products (Kozai T., 2015). Basing on the appropriate lighting conditions, other pressing studies uncompleted also including the improvement of luminous energy, the photosynthesis potential enhancement, and in-depth research on the responding mechanism between plants and light quality.

The objective of this study is to identify the ideal LED lighting “Recipes” (which includes lighting intensity and LED spectrum) for specific species and varieties, by determining the physiological and transcriptome characterization, which includes:

1). Submit a proper lighting recipe for commercial production by investigating the effects of LED lighting recipes on pak choi growth between different growth stages and varieties.

2). Investigate the effects of LED lighting on plants physiological reaction, especially the photoreaction function, stomatal conductance, PS II centre reaction, photosynthetic rate, and root development, to explore the physiological mechanism related to light conditions.

3). Using RNA-sequencing technique to determine essential genes that related to higher efficiency of photosynthesis and good quality of the plant growth under LED lighting recipes.

Chapter 2

Literature review

Chapter 2. Literature review

2. 1 Pak choi production and urban farming

Pak choi (*Brassica rapa subsp*. Chinensis), originate from China, now cultivated worldwide especially in east Asia. The appearance showed as obovate and 20~30 cm long leaves, with the color as dark green (Figure 2.1). It is rich in vitamins and minerals which helps to enhance the body's immune ability. Its shallow root distribution leads to higher transpiration rate, which normally needs higher air humidity and water content in soil, and because of its short growing period, pak choi requires loose and fertile soil. It can be produced all year round, but due to its cold tolerance, people often sow in autumn or winter to make use of idle farmland. With suitable conditions, the growing period of pak choi is generally 30 to 40 days, in winter it takes 100 days to harvest, and the yield per hectare is 1200 to 1500 kilograms (Keck A. S. and Finely J. W. 2004).

Figure 2. 1 *The appearance and characteristics of Pak choi (Brassica rapa subsp. Chinensis)*

At present, our traditional vegetable production is strongly affected by abnormal weather, water deficiencies and land shortages. Due to the limited natural resources, 90% of the global crop yields' growth is depending on higher production efficiency (Francesco N. 2007). For example, the United Nations predicted that nearly 70% of this population would be living in an urban environment by 2030. In the UK, 90% of the population lives in towns or cities (Sustainable Finance Geneva 2010). Consequently, to meet national food demands, a large proportion of their food relies on importing from other agriculture-oriented regions, including the ones with abundant natural resources, but economically backward. In the case, the food supply requires quantity and efficiency at the same time. Nearly 70% of the UK's vegetable and fruit supply comes from the EU and other countries and imported £8 bn of vegetables in 2016 (FAO 2017). There is concern that as Brexit food imports will become more expensive and there could be significant disruption to the food supply chain. Further to this, greenhouse gas emissions through the transportation of imported produce, as well as poor air quality in the UK is a significant public health issue. Therefore, urban agriculture, as a feasible way

to solve the contradiction between the concentration of labor force and less available resource in cities, is getting more and more attention from national governments. In Malaysia, the Agriculture and Agro-based Industry Minister Datuk Seri Ahmad Shabery Cheek indicated that there are around 11,000 urban farming communities around the country, and it aimed to create 20,000 urban agricultural communities by 2020. It is considered to be a government initiative program, to ensure a complete food supply chain and beef up food security in the country (Seri N. N. K. and Hidayatul A. A. 2018). Also, urban agriculture can precisely benefit low-income-countries by providing sufficient food, adequate nutrients, and income generation, it has the potential to improve nutritional security through direct availability of food in a cost-effective way (Golnaz R., Mad N. S., Zainalabidin M. 2015).

A typical urban agriculture model is vertical farming, which refers to a closed multi-layer production system with supplemental conditions such as artificial light, nutrient solution, temperature, and humidity control (Figure 2.2) (Kozai T. 2015). This indoor method with good air tightness and heat insulation, usually presents highly observable, controllable, predictable, and repeatable production capacity, and leads to maximum yield, higher quality, minimum input of resources and minimum emission of environmental pollutants (Kozai T. 2015). Compared with traditional agriculture model, the vertical farming, especially the mode with artificial light, has the following advantages: (1) Dispense the crop production with sunlight restriction, offering a wider range for agricultural site selection; (2) Extricate the plant growth from external environment and soil conditions; (3) Capable to achieve continuous production throughout whole year, resulting the productivity close to 10 times of rural field production; (4) Achieve high quality product by regulating the environment conditions (especially the lighting condition); (5) Improve food safety by actively prevent products from pesticides concern; (6) Guarantee swift and economical food transportation by locating urban agriculture sites closer to city area; (7)The

efficient utilization of resources can reduce environmental pollution (Kozal T. et al., 2015).

Figure 2. 2 *Typical vertical farming facility, Association For Vertical Farming, launched in 2013, USA.*

However, the urban agriculture's downside is still a struggling obstacle: the extremely high operation cost. In Japan's commercial vertical farming, the equipment depreciation (upfront investment), labor costs (or working time), and electricity costs respectively accounts for 26%, 21% and 18% of its annual sales, which caused only 30% of them can achieve profitability (Ijichi H. 2018. Kozal T. 2013). Among this, lighting electricity cost accounted for 75%~80 % of the total electricity consumption (Kozai T. 2016). In order to improve the energy utilization efficiency of lighting, we can either improve the photoelectric conversion efficiency of artificial lights—the newest LED lamp can achieve 3 mol/J (Kozai T., Uraisami K., Kai K. et al., 2019); search better-suited varieties

to artificial lights; or improve lighting conditions, including improved spectra, intensity, and photoperiod, to intensify the photosynthetic efficiency.

2. 2 Artificial light sources and LED lighting system

The application of artificial light sources in cultivated plants has been more than 150 years and has gone through four stages: incandescent lamps, open arc lighting, enclosed gas discharge lighting and Light-emitting diode (LED) solid-state lighting. At present, the field of plant supplementation mainly uses fluorescent lamps as artificial light sources. By changing the composition of the phosphors, the peak range of the spectrum of the fluorescent lamps can be changed, which is mainly distributed in the blue, green and yellow bands with less red spectrum. The photoelectric conversion efficiency of fluorescent lamps is about 20%, of which infrared consumption and thermal energy consumption account for 30% and 40%, respectively, and lead to the temperature increase of the lighting environment (Lin H., Wang B., Xu J. et al., 2014). Therefore, the fluorescent lamp’s usage always requires additional cooling equipment, which altogether further increases operating costs. This kind of heating illumination also affects the fluorescent lamps light efficiency, resulting in problems such as shortened life. On the other hand, unlike the adjustable light intensity and photoperiod, the fluorescence spectrum is continuous and cannot be precisely controlled to meet the needs of plants. Most of the inefficient bands cause additional photon escaping, resulting in a decrease in light energy utilization (Liu W., Yang Q., Wei L. 2012).

LED light is a semiconductor component, the core portion is a wafer composed of a p-type and an n-type semiconductor, and a transition layer between is referred to as a p-n junction diode. As electrons pass through the wafer, the negatively charged electrons move to and recombine with the positively charged hole region, which producing photons. The more significant the energy bandgap between electrons and holes, the higher the photon energy produced, and the photon energy corresponds to the generated spectrum. The blue light carries high energy, and the red light energy is relatively low. Different materials have different energy bandgaps, which can emit different colors of light (Zhao G., Pan Y. et al., 2013).

LED has the following advantages compared with traditional artificial light sources (Massa D., Kim H., Wheeler M. et al., 2008):

1) High light efficiency. The current white LED light efficiency has reached 220lm/w. In the future, the light efficiency of LED will surpass the existing artificial light source has become a consensus. Compared with the currently widely used fluorescent lamps, the efficacy of white LED can reach 5-8 times.

2) The spectral domain is narrow (Figure 2.3). The monochromatic light emitted by the high-power LED has a spectral range of ±50 nm. At present, the peak wavelength of monochromatic LED has covered the visible light band, and the appropriate wavelength band can be selected according to fit plant photosynthesis and photomorphology, thereby improving the absorption and utilization of light energy.

3) Environmental friendly. LED is a semiconductor light source, which is free from lead, mercury or other harmful substances compared with traditional artificial light sources.

4) Cold working temperature. Appropriate light spectrum selection can avoid the infrared and far-infrared bands and reduce the heat dissipation in the illumination, thereby achieving close-range lighting to the plants, improving space utilization, and reducing cooling costs, which is suitable for multi-tier cultivation mode.

5) Long lifetime. At present, the theoretical lifetime of LED is more than 50,000 hours. It is much higher than traditional artificial light sources such as fluorescent lamps, which can effectively reduce replacement and maintenance costs.

6) Flexible. The principle of illumination of the LED light source can easily meet different lighting requirements, including a more complex spectrum and more varied lighting schemes, which can effectively meet diverse plants' need in different growth stages. It also brings more accurate results and more possibilities for scientific research.

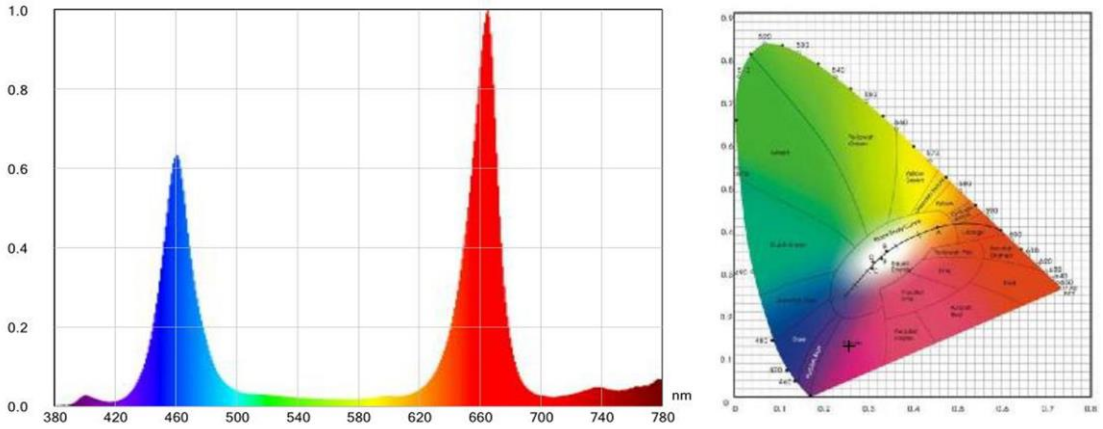


Figure 2. 3 *The spectrum and color temperature maps of LED lights for crops growth*

In conclusion, the LED light source is an ideal artificial light source for the current urban farming/vertical farming system. Its characteristics can effectively improve energy and space utilization and achieve more controllable growth and development of plants through more precise lighting conditions. Simultaneously, it can also effectively reduce running costs to bring considerable market meanings, which positively affects promoting and forming urban agriculture.

2. 3 The role of LED lights in vegetable production

Light energy is the dominating force for plant growth, as the light signal can induce biological development, photomorphogenesis, and the synthesis of secondary metabolites. Plants can receive electromagnetic waves which are between 280-800nm, and are especially sensitive to 400-700nm, which is called photosynthetically active radiation (PAR) (Jenkins I., Long C., Wade K. et al., 2001; Andreeva A. and Velitchkova M. 1998). Most of higher plants in nature require more completed spectrum for growth (Piao X. C., 2002; Chen X. L., Guo W. Zh. et al., 2014), but in commercial production the balance of cost-

yield is considered as the main factor. LED lights utilize lower energy cost due to their high photoelectric conversion efficiency. With the very narrow-band monochromatic light they presented, we can investigate the effects of the combined LED spectrum on plant growth and development at an acceptable cost.

Different light receptors in plants can sense separate light conditions such as intensity or wavelength. The absorption of chlorophyll is mainly dropped in red and blue light, while the light receptors in plants are known into three main categories: phytochrome is red - far red receptor; phytotropin and cryptochrome are blue light receptors. Thus, the research about the effects of monochromatic light was started with red and blue light (Huche-Thelier L. et al., 2016).

Red light is in the main chlorophyll absorption wavelength region, has significant contribution to the growth of crops. It has been reported that, 3 days before harvested, the lettuce with added 638 nm red light, 16 hours/day, 210 μmol/m2·s intensity, would present 28.50 % more total phenols content and 52.00% more soluble sugar content (Samuoliene G., Sirtautas R., Brazaityte A. et al., 2012). Supplementary 13 hours/day, 100-105 μmol/m2·s intensity, 650 nm red light, can dramatically increase the content of lycopene in tomato fruit, and significantly improves the sugar-acid ratio (Chen Q., Liu Q., Zhang K. et al., 2009). In Arabidopsis, add 6% red light can significantly increase the growth rate (Srinidhi V. et al., 2017).

Blue light is also in one of the main spectral absorption regions for photosynthesis and plays an important role in plant morphogenesis and

physiological metabolism. It has been reported that, blue light can significantly inhibit the elongation of plant hypocotyl and shorten the length of internodes, this result has been found in Arabidopsis Thaliana (Zhang Y., Yu Q. et al., 2017), cucumber seedlings (Ma D. et al., 2016) and lettuce seedlings (Lee I. 2010). Compared with the blank control, blue light treatment can significantly increase the content of phenolic compounds and antioxidant capacity (FRAP) in pea sprouts (Liu K. et al., 2016). With the increase of blue light intensity, the leaf color of red lettuce became significantly deeper (Singh S. et al., 2017).

Compared with monochromatic lighting, the research of composite lighting ("Lighting Recipe") has been more concerned. Soluble sugar, as the primary product from photosynthesis, is also the basic material of other metabolic processes, and the glycolysis process it involved can provide the energy for other synthesis of macromolecular compounds (Ma et al., 2010). Study has shown that, the beet grew under composite LED light (red and blue) had the highest sugar and starch content in root, compared with other monochromatic lighting treatments (Singh et al., 2017). The reason about these different effects of LED lighting recipes is that the spectrum can directly affect the accumulation of carbohydrates, and it may induce the expression of relevant photoreceptors in plants, to regulate the pathway of sucrose metabolizing enzyme, thus accelerating the sugar synthesis (Feng X. et al., 2011).

And previous research on the LED lighting recipes has also showed remarkable diversity between different species. Lin K. et al. (2013) reported hydroponically grown lettuce treated by fluorescent lamp can provide higher biomass and soluble sugar content compared with monochromic red or blue LED light, but Li H. M. et al. (2013) reported that red and blue LED lights treatments had greater effects on the yield of *Brassica napus*, compared with fluorescent lamp. Yang

Y. T. et al. (2010) compared the ratio of red /blue LED light as 4:1, 6:1, 8:1, and 10:1, respectively for seedling growth in sweet potato and the results showed that the 8:1ratio of red /blue light inhibited excessive growth，reduced plant water content and increased root-shoot ratio. In contrast, Nhut D. T. et al. (2003) reported the banana seedlings under 8:2 of red to blue ratio had higher shoot and root fresh weight than those receiving other treatments. They also found that under LED light with the 7:3 ratio of red/blue, the leaves number, root number, root length, fresh weight and dry weight in strawberry seedling reached the peak, and this remained after transplanting. These differences are mainly caused by the differences of photoresponse between species and varieties.

2. 4 Effects of LED lights on photosynthesis

Light is one of the most critical environmental factors in crop production; it actively participates and regulates plants' photosynthesis. Studies of proposition have attracted many researchers, mainly found into following aspects: (1) Light is the necessary condition for plant photosynthesis, which provide the energy; (2) The synthesis of photosynthetic pigment needs the light participation; (3) Light spectra, light intensity and light period can all affect the stomatal movement; (4) Many crucial enzyme activities in photosynthesis process were affected by light conditions.

The absorption of different light spectrum in plant photosynthesis process is not similar. For example, Mccree K. et al., (1972) investigated 22 kinds of crops under different environmental conditions (including temperature, carbon dioxide

concentration and leaf orientation). The quantum yield curve results showed the peak presented in red and blue light area, which indicated that red and blue light is the most effective for photosynthesis. The reading of blue light is 30% less than red light, which means red light is more effective at photosynthesis than blue light (Mccree K. et al., 1972). Katsumi's study came to the same conclusion: some of the blue light was absorbed by beta-carotene, which showed ineffective for photosynthesis. Thus, red light participates in photosynthesis more effectively than blue light in production of photochemical product (Katsumi I. 1976).

Kim et al. treated chrysanthemums with different LED light treatments, which referred to 100% blue light, 100% red light, 50% red light +50% blue light, 50% blue light +50% far-red light, 50% red light +50% far-red light, compared with fluorescent lamps as control. The results showed that the net photosynthetic rate was the highest under the treatment of 50% red light + 50% blue, and the lowest under 50% blue light and 50% blue light + 50% far-red (Kim H. H. et al., 2004). As an essential factor affecting photosynthesis, light intensity was also explored by researchers. Johkan et al., (2012) treated red lettuce under different light intensity (100PPFD, 200PPFD and 300PPFD) and different spectrum (510nm, 520nm and 530nm), and the results showed that the photosynthetic rate increased with the light intensity increasing and showed the highest reading at 510nm under all three intensity (Johkan M. et al.,2012). Muneer et al., (2014) treated lettuce with six changed intensity and spectrum LED lights: 70 μmol /m^2s and 180 μmol /m^2s green lights, 88 μmol /m^2s and 238 μmol /m^2s red lights, 80 μmol /m^2s and 238 μmol /m^2s blue lights, respectively. The results showed that the net photosynthetic rate presented the highest reading under 238 μmol /m^2s blue light treatment. Under the same spectrum (green light), the net photosynthetic rate with high light intensity (180 μmol /m^2s) was significantly higher than that with low light intensity (70 μmol /m^2s) (Muneer S. et al., 2014).

2. 5 Effects of LED lights on chlorophyll fluorescence

The photosynthesis in leaf can be roughly divided into photoreactions and dark reactions, which refers to photolysis of water for ATP synthesis, and the fixation of carbon dioxide and the reduction of C3 compounds. (Frechette E. et al., 2016). The history of the chlorophyll fluorescence kinetics phenomenon is more than 80 years since Kautsky and Hirsch first observed with naked eye in 1931. However, the application of chlorophyll fluorescence kinetics as a technique for photosynthesis research has been the case for only about 30 years (Zhang S. R. 1999). To unravel the kinetics steps, the chlorophyll molecule's main structure is a porphyrin ring centered on Mg, which formulate a ring-mounted carbon skeleton structure that possesses the property of light absorption. Once the chlorophyll molecule located on the thylakoid membrane absorbs light, Mg's electrons undergo a transition and generally, the energy of one photon will only cause one-time electron transition. This high-energy electron will pass through the chlorophyll molecules until the receptor located at the founded photoreaction center and the energy be transferred out. The energy that is not delivered smoothly will be emitted in the form of heat and fluorescence (Christopher G. et al., 2017). The relationship between the three processes can be shown as:

$$Fluorescence + heat + photochemistry = 1$$

Thus, for estimating the number of electrons used for photosynthesis, the varying lighting conditions were used to stimulate different closure level of the photoreaction center (Photosystem II), to obtain the amount of fluorescence

yields in various states, for calculating the actual electrons usage in photochemical reactions (Maxwell K. and Johnson G., 2000). The chlorophyll fluorescence kinetics technique has a unique role to detect light system absorption, transmission, dissipation, and distribution during photosynthesis. (Genty B. et al., 1989; Schreiber U. et al., 1994).

The parameter F_v/F_m was measured after dark adapted, with this condition the PS II (Photosystem II) was fully opened, showing us the maximal PS II efficiency. Generally speaking, this parameter changes insignificantly, only decreased if the plant is under stress conditions. Meanwhile F_v'/F_m' is measured without dark adapted, and PhiPS2 showed the actual photochemical efficiency of PS II in the light. Zhou J. et al., (2014) reported that the F_v/F_m of tissue cultured *Anoectochilus roxburghii* under blue light treatment was higher, while those under red light treatment be found relatively low. Wen J. et al., (2016) compared the development of *Dendrobium officinale* under various lighting set, including red light, blue light, yellow light, red + blue light, and red + blue + yellow light treatments. The results for the experiment indicated the fact that only the red + blue brings to the most positive impact to plants energy absorption, transformation, transmission, distribution, and the dissipation of PS Ⅱ reaction. Xu K. et al. (2005) also indicated that F_v/F_m and PhiPS2 values of cucumber seedlings were significantly higher under the red + blue LED lights cultivating condition.

2. 6 Light-harvesting complex and signal transduction in photoreceptors

In higher plants, photosynthesis mainly occurs in the thylakoid membrane of chloroplasts, with four primary types of membrane protein complexes: photosystem II, photosystem I, b6/f complex and ATPase complex. The light-harvesting complex is mainly distributed in PSII and PSI. Its principal function embodies four aspects: capturing and transmitting light energy, light protection maintaining the thylakoid membrane structure. The peripheral light-harvesting antenna in PSII (LHCIIa, LHCIIb, LHCIIc and LHCIId) is the leading research object as most energy source comes from LHCIIb, and its chlorophyll content accounts for 50% of the total pigment on the thylakoid membrane. In the plant leaves adapted to low light intensity conditions, the peripheral photosystem II complex combines two main light-harvesting complexes LHCII (divided into S-LHCII and M-LHCII according to the affinity) and three minor light-harvesting complexes: CP29, CP26 and CP24. These light-harvesting complexes are combined with the photosystem II to form a C2S2M2 super complex, which enables efficient capture of light energy and energy conversion under low light conditions (Su X. D. et al., 2017). While under strong light conditions, plants need to dissipate excess energy to protect photosynthetic organs from damage. The most important one is non-photochemical quenching that relies on proton gradients, always accompanied by aggregation of LHCII as the main site, which adjusts the absorption and transmission of excitation energy (Heijde M. and Ulm R., 2012). The plants sense light signals through different photoreceptors and then regulate various plant growth and development responses by corresponding signalling pathways. Five kinds of photoreceptors are founded with distinctive functions, listed as following: The phytochrome can sense red or far-red light from 600nm to 750nm; the cryptochromes can sense blue light and UV-A from 320nm to 500nm; the phototropin can also sense blue light, but mainly regulates the plant phototropism, chloroplast movement and seedling

growth. The UV-B and blue-green photoreceptor still require further study (Ma, D. 2007; Cashmore A. R. et al., 1999; Liscum E. and Briggs W. R. 1995; Kagawa T. et al., 2001; Somers D. E. et al., 2000; Jenkins G. I. 2009; Heijde M. and Ulm R. 2012).

The phytochrome is a hemispherical photoreceptor (Vierstra R. D. and Zhang J. 2011). There are two main types: a chemically stable red light absorbing type (red absorbing form, Pr) that can transform into an active far red absorbing type (far-red absorbing form, Pfr) after sensing the light signal, and Pfr can be degraded to Pr after ubiquitination (Casal J. J. 2013). The apoprotein of phytochrome is encoded by the genes of PHYTOCHROME A (PHYA), PHYB and PHYC (Mathews S. 2006), and PHYB is the most important type of photoreceptor. The PHYB in Arabidopsis can sense the shading conditions and affect the morphological construction of the plant, including the elongation of stems and petioles, the establishment of apical dominance and the generation of erect leaves (Ballare C. L. 2009). On the other hand, PHYA is considered to be a good photoreceptor that responds to changes in irradiance, as Yanovsky et al., 1995 reported that PHYA played the vital role in light-dark formation of Arabidopsis seedlings when affected by low light stress.

Cryptochrome is classified into three species in Arabidopsis: CRY1, CRY2 and CRY3 (Lin C. et al., 1996). Members of the CRY gene family are species-specific, such as tobacco (Nicotiana tabacum) and rice (Oryza sativa) have two CRY1-like family members (Perrotta G. et al., 2000), while pea (Pisum sativum) has two CRY2 family members (Platten J. D. et al., 2005). At the same time, the effect of blue light on CRY gene expression is also species-specific, the CRY2b gene in peas is significantly down-regulated after blue light treatment, while the expression level of CRY1 in rapeseed (*Brassica napus*) is significantly

up-regulated (Platten J. D. et al., 2005).

The phototropin is a type of serine/threonine protease that is activated by blue light and UV-A (Christie J. M. 2007). There are two different types of phototropin in Arabidopsis, PHOT1 and PHOT2, both located in the plasma membrane, which are detached from the plasma membrane after activation by UV-A and blue light and induce signal transduction after binding to the target protein (Casal J. J. 2013). The gene expression of PHOT receives blue light regulation, which can promote the expression of PHOT2 and inhibits the expression of PHOT1 (Labuz J. et al. 2012). PHOT is involved in the photosynthetic performance of plants and chlorophyll accumulation and stomatal opening in Arabidopsis (Esmon C. A. et al. 2006; Ma L. et al. 2011).

When the plant senses the light signal, it prime activates photoreceptor proteins' expression to respond to different wavelengths of light (PHY, CRY, UVR8, etc). These photoreceptor proteins later physically interact with the negatively regulated COP1 protein, and the number of upstream photoreceptor proteins increasing leads to the increasing in consumption of COP1, which hinders the degradation of proteins such as HY5, HFR1 and LAF1, promotes their accumulation, thereby regulating downstream structural genes and affecting the photomorphogenesis of plants (Lau O. S. and Deng X. W. 2012; Stracke R. et al., 2010).

2. 7 Using transcriptome to investigate the effects of LED on plant growth

At present, research on light-harvesting and light signaling pathways is mainly concentrated in the model plant Arabidopsis thaliana. In order to expand the diversity of plant samples, the *Brassica rapa* as a representative of the origin Brassica genome has big potential to be a model of genomic research for its family (Chaobo T. 2013). Although the light regulations of photosynthesis with enzymatic properties of individual enzymes are well studied, a fundamental regulatory mechanism at large scale gene expression mapping is still very limited. However, the publication of Brassica rapa Chiifu-401-42 whole genome in 2011 gave us a great opportunity to further identify and investigate the comprehensive transcriptome characteristic on a genome-wide scale (Wang X. et al. 2011).

In additions, the novel High-throughput RNA sequencing technology has become effective to genomic research. With millions of generated cDNA reads from experiments, RNA-seq approach is capable to provide genome-scale transcriptional profiling, benefiting the transcriptome's comprehensive analysis with plenty of superiority. To be specific, first, RNA-seq will quantify the gene expression level, especially it can sensitively detect low expressed genes (Mortazavi A. 2008). Second, it will allow the researchers to improve gene annotations, to discover novel genes and transcripts by extending transcriptional boundaries (Ozsolak F. 2011). Third, RNA-seq will be able to identify gene function and regulation by further gene regulatory network analysis and survey alternative splicing (AS) events on single nucleotide resolution and other transgenic approaches (Wilhelm B. T. 2008) (Figure 2.1).

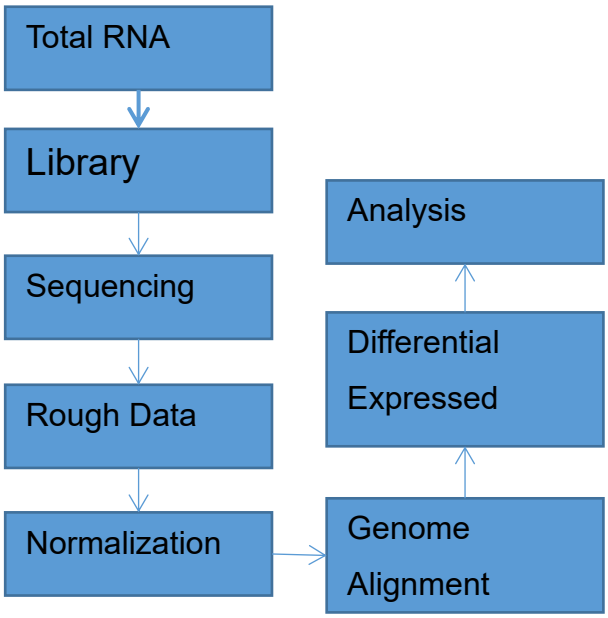


Figure 2.4 *The technological process of High-throughput RNA sequencing*

With these benefits, RNA-seq is qualified to be an ideal research approach for investigating plant changing transcriptional response to the diverse lighting conditions. Mehmet T. et al. (2016) used RNA-seq to detect the regulation of red and blue light on cellular functions, cell division, and cell repair processes in red alga. Chunxia L. et al. (2017) also adopted RNA-seq to provide integrated insights of chlorophyll synthesis, chloroplast development and carbohydrates assimilation in grape plantlets being treated by blue, green and red LED lights. With a large number of genes been detected to participate the metabolic pathways of glucan, starch and sucrose, while up/down-regulated by different LED treatments. The observation may unravel some fact of how the grapes' nutrition level increase under certain LED light spectra. With the genetic mechanisms analysis of Norway spruce seedling treated by different light spectrum, Ouyang F. et al. (2015) found that the red light may improve stem growth by promoting the biosynthesis of gibberellic acids, and the blue light may promote the tolerance by increasing the content of flavonoid, phenylpropanoids and other secondary metabolite, which consequently reduce the primary metabolites.

Chapter 3

Materials and Methods

Chapter 3: Materials and Methods

3. 1 Experimental Sites

Due to the Split-site project proposal, the experiments were running in two campuses of the University of Nottingham: the experiments in UK campus were conducted in B05 lab, Gateway Building, Sutton Bonington campus, and the experiments in Malaysia campus were conducted in Crops for the Future Research Centre lab. All experiments were performed in closed growth chamber.

3. 2 Materials

The tomato (*Solanum. Lycopersicum*) and pak choi (*Brassica rapa, Chinensis*, Green revolution F1) was chosen for the UK campus experiments. Also, there were four varieties of pak choi selected for the experiments in the Malaysia campus, which were 802 Bushido and Kungfu 2, supplied by Green World Genetics; 702 Warrior and Green Hill, supplied by Leckat Seeds. The selected varieties were all commonly consumed in their region. The seeds were stored a 4 ^{o}C fridge in sealed containers to keep cold and dark.

3. 3 Preliminary experiment

The preliminary experiments were conducted after the lighting devices installed to test the device's function. Pak choi and tomato were germinated under LED and fluorescent lights. while the seeds were sowed in the whole tray with each plug containing three seeds, then followed the methods described in section 3.4 and 3.5. After seven days of germination, the stability of LED lighting devices was confirmed as no obvious faults observed, the PPFD was stable, and the adjustable light recipe can be functioning. Also, the seedling of pak choi and tomato were calculated for germination rate.

3. 4 Nursery management

The preliminary experiments had shown us that the pak choi seeds performed the semblable reaction to disparate lighting during their germination. To avoid further error between the samples, all germination process was accomplished under fluorescent lamp, with the seeds be sown in the whole tray, covering plastic wrap to reduce water transpiration. Levington M3 High Nutrient Potting Compost (Everris) was chosen for all growth stage substrate, showed as Figure 3.1. As following, only the sprout with the best growing status chooses retained after being germinated, maintaining one seedling in each slot. Growth conditions in this experiment as temperature, CO_2 concentration and relative humidity were controlled by STS Refrigeration System (Storage Technology Solutions Ltd), with set data as 12 hours lighting length, 12 hours dark cycle with the temperature of 24 ^{o}C for daytime and 20 ^{o}C for dark time.

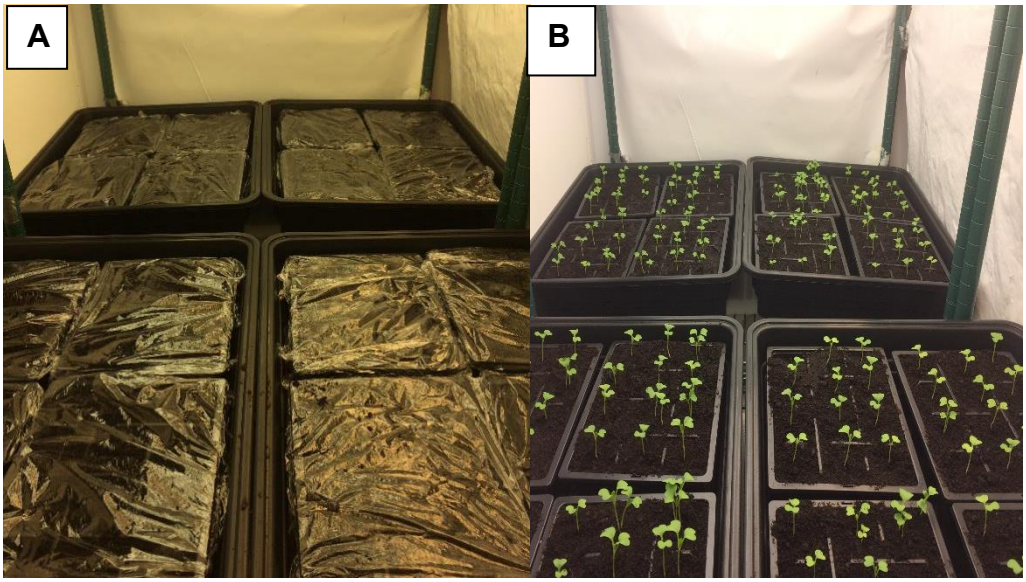


Figure 3. 1 A *The first day of germination and* ***B*** *the 6th day of germination (Pak choi).*

3. 5 Plant establishment

After 6 days of germination, the healthy seedlings were transplanted in to 10cm diameter pot filled with potting compost (Levington M3 High Nutrient). Due to the limited space under LED panel, each treatment had 6 pots as 6 replicates. All pots were placed in tray and irrigated every day for 30 days (Figure 3.2). In the circumscribed growth scope, the plants were clockwise rotated every day to ensure balanced and unified light absorption. For tomato's growth, its plant height was higher than other leafy vegetables, so the distance between the plants and LED lighting panels were adjusted longer. 5 days as a routine, it should be checked that the highest point of tomato canopy was receiving the same photosynthetic photon flux density (PPFD) as others.

Figure 3. 2 *The pak choi samples after transplanting. The place of each pot was clockwise switched every day to make sure the light distribution was uniform.*

As all experiments were processed in closed environment, there were no pesticide or chemical sprayed. Also due to the short growth period of pak choi and rich nutrition in the substrate, there were no additional fertilizer added during the experiment.

3. 6 Light treatments

For the experiments in UK campus, two artificial lighting systems were used:

1) Fluorescent light panel, assembled by 6 fluorescent tubes (54 wattage per tube), supplied by Unigro Fluorescent Lamp, light model 12/6N.

2) LED light panel, each panel contained ten tubes (PHILIPS Green power research module), with selected wavelength: deep red (650nm~670nm, ten wattages); blue (455nm~485nm, 14 wattages) and far-red (725nm~750nm, ten wattages), each tube refers to on kind of single light.

The spectrum scanning of each tube wavelength has shown in Figure 3.3. The peak of the blue light wavelength was at 452nm, and the peak of the red light wavelength was at 658nm. The wavelength of fluorescent lamp sets mainly refers to orange light (610nm), green light (553nm), and blue light (439nm).

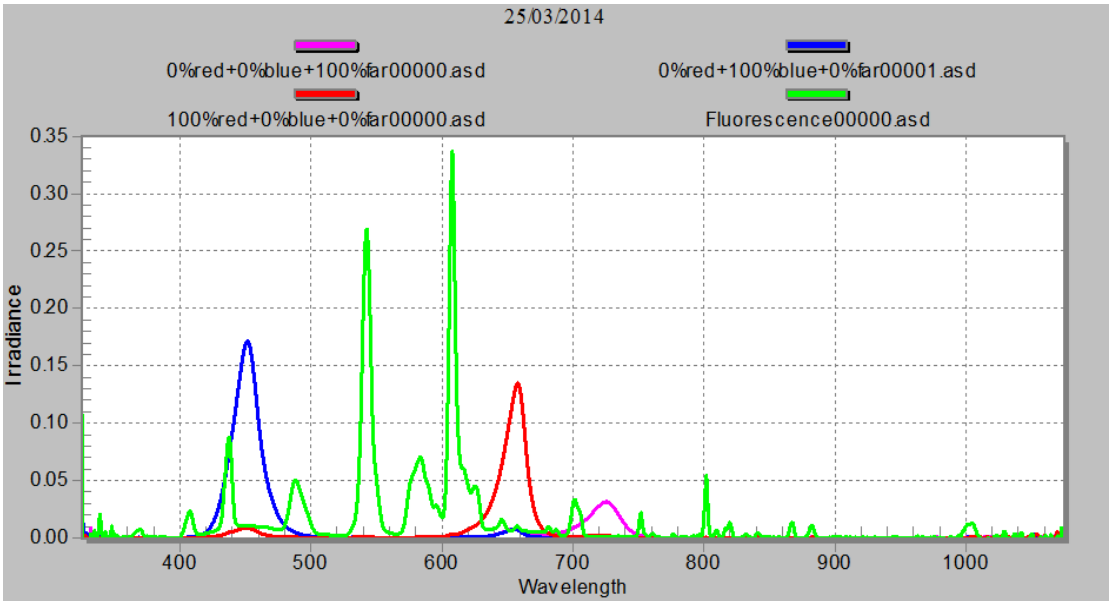


Figure 3. 3 The spectrum scanning of light source wavelength. Effective measurement range is from 350nm to 900 nm.

From preliminary experiments, the intensity was chosen as 130μmol/m^2s. However, the Fluorescent lamp was unable to change the intensity. Thus, the PPFD was controlled by changing the distance between the lamp and the plants, which was 31.2cm. The PPFD was measured by Skye Instruments spectra sense RS 232.

The “Light recipe” of LED lighting, which refers to the PPFD and ratio between red/blue/far-red light from each LED panel, was adjusted by control units designed and made by Manor Maintenance Electrical Services Ltd, UK. Each LED panel contained ten tubes as one chamber and sealed by a plastic sheet to avoid light contamination from others (Figure 3.4).

Figure 3. 4 A: *Single LED lighting panel, with 10 tubes from left to right: red, red, blue, red, red, far-red, red, blue, red, red, respectively.* ***B:*** *The full view of LED lighting chamber, the sheet was opened for watering.*

With controlled lighting units, the light recipes have been used for different experiments:

Experiment 1: Effect of LED light intensity on growth and yield of pak choi

The pak choi was grown under LED lighting for 30 days compared with fluorescent lamp treatment with six replicates. The lighting recipes of LED treatments were set as red/blue: 70% / 30% and changed light intensity from 100 to 200 μmol/m^2s, detail showed in Table 3.1.

Table 3. 1 *The different LED intensity treatments used in experiment 2.*

Treatment	Recipe (Red : Blue)	Intensity(μmol/m^2s)
CK	/	200
Recipe H (High intensity)	70% / 30%	200
Recipe M (Medium intensity)	70% / 30%	150
Recipe L (Low intensity)	70% / 30%	100

Experiment 2: Effects of LED recipes on growth and yield of pak choi and tomato

The pak choi and tomato were grown under different LED lighting recipes for 30 days with six replicates. The establishment of tomato growth was following the same procedures in section 3.4-3.5. The fluorescent lamp was used as a control (CK). As the space of the growth chamber was limited, we had to reduce the intensity to make sure that the canopy of the tomato can receive balanced PPFD. The intensity was set to 103 μmol/m^2s, pak choi and tomato were grown under different LED recipes shown in Table 3. 2.

Table 3. 2 *LED recipes used in experiments 2*

Treatment	Red light proportion (%)	Blue light proportion (%)
CK	/	/
Recipe 1	100	0
Recipe 2	77	23
Recipe 3	73	27
Recipe 4	70	30
Recipe 5	62	38
Recipe 6	0	100

Experiment 3: Growth and yield of Malaysian pak choi varieties grown under fixed LED recipes

There were four Malaysian common varieties chosen for this experiment: 802 Bushido, Kungfu 2, 702 Warrior and Green Hill, which were all F1 hybrid dwarf pak choi varieties. The experiment was conducted in A1000-Growth chamber, Conviron, supplied by Crops For the Future research centre. In order to ensure the samples were processed under controllable conditions, the experiment was conducted into two chambers, with fluorescent light treatment and fixed LED spectrum, respectively, each treatment has 3 replicates. The LED panel in this experiment contained 3 tubes, refers to red, blue, and red, orderly. Total PPFD was set to 130 μmol/m^2s by changing the distance between lamp and plants, and the ratio of red/blue was set to 7/3, showed in Figure 3. 5

Figure 3. 5 *Selected samples of four varieties were transferred to different light treatments.* ***A:*** *the fixed LED light treatment and* ***B:*** *the fluorescent light treatment as control.*

3. 7 Data collection

3. 7. 1 Plant height

The length of the main stem of pak choi was considered plant height. It was measured every three days after transplanting. The hypocotyl was considered plant height for the early-stage experiments where the stem not sufficiently developed.

3. 7. 2 Leaf traits

The leaf number was measured every three days after transplanting. Every leaf that was fully unfolded from the shoot was counted. After 30 days, the samples were harvested by secateur; all leaves were cut and cleaned, measured by Li-cor Model 3100 area meter for leaf area. As the petiole of pak choi was fully developed and considered an edible part, it was also covered in the measurements.

3. 7. 3 SPAD and ACI measurement

The 3rd fully expanded leaf was used for chlorophyll (SPAD meter) and anthocyanin (ACI) measurements. As the third leaf was the first developed one after transplanting, it has been considered a suitable leaf for measurements (Erik H. M. et al., 2005). It was recorded as an average of six measurements by SPAD-502 chlorophyll meter (Konica Minolta, UK) and ACI meter, which measures the absorption of wavelengths corresponding to chlorophyll and anthocyanin, considered as the relative content.

3. 7. 4 Chlorophyll concentration

The Post-harvest chlorophyll content was detected with acetone extraction: Using 0.1g actual leaf sample and cut to strips, put into 10ml of 80% acetone for 24 hours extraction under dark (vibrated every 6 hours), then measured the absorbency by spectrophotometer (CE 2041, CECIL) with 645nm, 663nm and 470nm as A645, A663 and A470. The Arnon formula could calculate the chlorophyll concentration (mg/L):

Chlorophyll a concentration: C_a=12.7(A663) - 2.59(A645);

Chlorophyll b concentration: C_b= 22.9(A645) - 4.67(A664);

Carotenoid concentration: C_c= (1000(A470) - 3.72C_a-104C_b)/229

Total chlorophyll concentration: C_t= C_a+C_b

3. 7. 5 Fresh weight (yield) and dry weight

After 30 days of the growth, the fresh sample was harvested, overground parts were cleaned with water and air-dried. The fresh weight was weighting by Mettler Toledo XS6002S electronic scales. All the samples were dried in an oven at 65 ^{o}C for 48 h for dry matter measurements.

3. 7. 6 Root morphology

The roots of pak choi were soaked in water for 30 mins, then removed all the soil and scanned by EPSON Flatbed Scanner. WinRhizo 2013e analysed the root structure image, including total root length, total surface area, average diameter, total root volume, and the number of tips, forks and crossing.

3. 7. 7 Gas exchange and chlorophyll fluorescence

The photosynthetic physiology was measured by Li-6400XT Infra-Red gas analyser (IRGA) (Licor Biosciences, USA), which included maximum photosynthetic rate, stomatal conductance and chlorophyll fluorescence. The measurements started after 1-hour light activation on the leaves in the morning, the flow rate was set to 400 μmol/s, leaf temperature within the Licor unit was

set to 25 degrees. The fluorescence measurement setting has been shown in Table 3.3.

After light-adapted measurements, the measured leaves were marked and covered by foil for 50 mins dark adaption, to ensure that all photosystem II in the chlorophyll molecules is oxidised. Then carefully remove the foil for chlorophyll fluorescence measurements under dark. The setting has been shown in Table 3.4.

All measurements were taken from the 3rd fully expanded leaf, the leaves were exposed in the Licor unit for a minimum of 5 minutes to make sure stabilise the readings.

Table 3. 3 *Chlorophyll fluorescence measurements setting for light adapted measurements*

Measurements	Intensity	5
	Modulation	> 0 kHz
	Filter	1
	Gain	10
Flash	Duration	0.8 s
	Intensity	8
	Blue	No change
	Modulation	> 0 kHz
	Filter	50 kHz

Table 3. 4 *Chlorophyll fluorescence measurements setting for dark adapted measurements*

Measurements	Intensity	1
	Modulation	0.25 kHz
	Filter	1
	Gain	10
Flash	Duration	0.8 s
	Intensity	600
	Blue	No change
	Modulation	> 0 kHz
	Filter	50 kHz
Dark	Duration	6 s
	Far red intensity	8
	Far red per time	1 s
	Far red post time	1 s
	Modulation	0.25 kHz
	Filter	1 Hz

3. 8 RNA extraction and RNA sequencing

3. 8. 1 Sample collection and RNA isolation

After harvesting the pak choi, the fresh leaves were collected and immediately frozen in liquid nitrogen. Samples from each treatment were prepared as three replicates: 100% blue LED light treatments (B1, B2，BN)，100% red LED light treatments (R1, R2, RN), 70% red / 30% blue LED light treatments (M1, M2, MN) and fluorescent light treatments as control (CK1, CK2, CKN). Total RNA was isolated and extracted by TRIzol reagent (Invitrogen Scientific, Inc., USA) and Qiagen RNEasy (RNeasy Mini Kit, Qiagen, USA), as the leaves of pak choi are covered by wax, which makes the samples inadequately touched by the reagent. Thus, the procedure was modified; see details in appendix 1. The RNA integrity was confirmed by using the 1% Agarose gel electrophotometer. The NanoDrop micro-ultraviolet-visible spectrophotometer and Agilent Bioanalyzer (Agilent Technologies, Inc., USA) were used to quantify the total RNA content and determine its quality.

3. 8. 2 RNA sequencing and reads alignment

The mRNA obtained from total RNA was isolated and converted to cDNA and amplified by PCR according to the Illumina RNA-seq protocol (Illumina, Inc.,

USA). Sequence reads were generated using Illumina NextSeq500 at the Deep Seq Centre for Genetics and Genomics, The University of Nottingham.

Afterwards, CLC Genomics Workbench 8.0 was used for normalization (PCA for RNA-seq, Differential Expression for RNA-Seq) by TMM (trimmed mean of M values) method of Robinson and Oshlack, 2010:

https://genomebiology.biomedcentral.com/articles/10.1186/gb-2010-11-3-r25

Ultimately, the sample genes were mapped to the reference genome. Parameter used as Table 3. 5.

3.9 Statistical analysis

The collected data were analysed using variance procedure (ANOVA) of two-way ANOVA in a completely randomized design (CRD) experiment. The significant difference between the treatments was analysed using Tukey's and Duncan test for mean separation, and the P-value of <0.05 was consider significant. In gene expression, RPKM and log2 fold-change was used to present expression level.

Table 3. 5 *The mapping parameter with CLC Genomics Workbench 8.0*

Reference sequence	BrapaFPsc_277_v1 (Genome)
Gene track	BrapaFPsc_277_v1.3 gene. gff3_Gene
mRNA track	BrapaFPsc_277_v1.3 gene_exons. gff3_mRNA
Mapping type	Also map to inter-genic regions
Mismatch cost	2
Insertion cost	3
Deletion cost	3
Length fraction	0.8
Similarity fraction	0.8
Global alignment	No
Auto-detect paired distances	Yes
Strand specific	Both
Maximum number of hits for a read	10
Count paired reads as two	No
Expression value	Total counts
Calculate RPKM for genes without transcripts	No
Create report	Yes
Create list of unmapped reads	Yes

Chapter 4

Results

Chapter 4: Results

4.1 The physiological responses and LED lighting recipes optimizing

The purpose of Section 4.1 is to identify the physiological responses of the different experimental subject against diversified LED lighting recipes, explicitly are the changed lighting wavelength and intensity for different species at different growth stages. Most higher plants in nature require a much complete spectrum for growth (Piao, 2002; Chen et al., 2014), but the balance of cost and yield is the main factor considered in commercial production. LED lights can operate with more economical energy cost due to their high photoelectric conversion efficiency. Additionally, they can provide very narrow-band monochromatic light that allows us to investigate the effects of the combined LED spectrum on plant growth and development with acceptable cost.

4.1.1 Phenotypic analysis among different species, yield and yield components

Generally speaking, the LED-lighting recipes analysing should be processed with three perspectives: the spectrum (the ratio between different wavelengths), the intensity and the lighting duration. Significantly, the spectrum and intensity

are the key factors for manipulating plants yield through photosynthesis controlling. By observing and comparing a plant's lighting condition with the yield components and morphology features, we could learn to achieve a precise production with subtle adjustments. The investigation was conducted in tomato: represented perennial herbaceous plant with more fully developed stem and canopy structure (Table 4. 1. 1); lettuce: with dwarf and heading leaf development (Table 4. 1. 2); and pak choi: dwarf structure from Cruciferae family with the potential to become future model crops (Table 4. 1. 3).

Table 4. 1. 1 *The physiological features of post-harvest tomato seedling, treated by different LED lighting recipes, compared with fluorescent treatment as control (CK).*

Treatment (Recipes)	Plant height (mm)	Fresh matter (g)	Dry matter (g)	Leaf area (cm^2)
CK	200.32±6.92d	18.13±0.42b	3.22±1.26b	20.36±1.78a
Recipe 1	311.04±8.23a	13.53±1.37d	2.26±0.33c	15.36±3.06c
Recipe 2	293.71±7.11a	15.61±1.89c	2.37±0.44c	14.98±4.25c
Recipe 3	268.02±4.54b	19.54±2.42a	4.05±0.26a	20.81±1.81a
Recipe 4	264.54±7.93b	17.73±2.65b	3.14±0.15b	17.91±0.98b
Recipe 5	258.73±6.42b	16.77±1.24c	2.64±0.16c	13.17±2.78d
Recipe 6	254.21±2.14c	13.42±0.46d	1.89±0.21d	13.57±2.56d

**Within a column, means followed by different letters are significantly different at p≤0.05, n=3; values after ± are standard deviation values. Recipe1=100% red: 0%blue; Recipe 2=77% red: 23% blue; Recipe 3=73% red: 27% blue; Recipe 4=70% red: 30% blue; Recipe 5=62% red: 38% blue; Recipe 6=0% red: 100% blue; CK: Fluorescent lamp.*

The tomato seedling showed sensitive reaction to the changed LED recipes. In

particular, the morphology was significantly ($P<0.05$) regulated. All LED treatments showed higher plant height compared with CK, and this decreased with the increasing proportion of blue light. This indicated that the blue light showed negative effects on stem elongation. On the other hand, although the CK had the lowest plant height, it showed positive results in both fresh matter, dry matter and leaf area (Table 4. 1. 1) From external formation of canopy it was observed that tomato grown under fluorescent lamp presented pyramidal structure and the LED treatments tend to show a cylindrical structure that appeared to be fragile and exhibit lower potential for later growth (Figure 4. 1. 1).

Figure 4. 1. 1 *The canopy of tomato seedlings under different treatments. From left to right refers to CK, recipe 1, recipe 4 and recipe 6, respectively. The recipe 6 as 100% blue proportion showed strong stress to tomato seedling.*

Among the LED treatments, recipe 3 indicated the dramatically increased fresh matter, dry matter and leaf area compared with the others. Recipe 1 (monochromic red light) and recipe 6 (monochromic blue light) significantly ($P<0.05$) stressed the tomato growth as a rapidly decreasing in the total yield. An opposite tendency between plant height and biomass from CK was observed, suggesting that the full-scaled spectrum had a positive effect on plant morphology. In general, with relatively lower light intensity, the LED recipes as fragmentary spectrum had further stressed tomato growth as negative accumulation results had been observed compared to CK. Although specific red/blue ratios can alleviate light stress, it still caused spindly growth and showed a lack of development and flourishment.

Table 4. 1. 2 *The post-harvested lettuce physiological features which were treated by changed red/blue ratio for 30 days*

Treatment (Recipes)	Plant height (mm)	Fresh matter (g)	Dry matter (g)	Leaf area (cm^2)
CK	4.35±0.02b	14.525±0.17a	0.92±0.01b	27.43±1.03a
Recipe 1	4.52±0.01b	12.045±0.27c	0.75±0.01c	22.13 ±0.88c
Recipe 2	4.55±0.05b	13.346±0.18b	0.89±0.02b	26.97±1.59a
Recipe 3	4.47±0.02b	14.569±0.18a	1.15±0.01a	28.11 ±2.78a
Recipe 4	4.65±0.01b	13.532±0.14b	0.91±0.02b	27.36± 1.68a
Recipe 5	4.52±0.04b	13.362±0.23b	0.87±0.01b	27.76 ±1.56a
Recipe 6	5.35±0.08a	13.574±0.13b	0.76±0.02c	24.43 ±0.83b

**Within a column, means followed by different letters are significantly different at P ≤0.05, n=3; values after ± are standard deviation values. Recipe1=100% red: 0%blue; Recipe 2=77% red: 23% blue; Recipe 3=73% red: 27% blue; Recipe 4=70% red: 30% blue; Recipe 5=62% red: 38% blue; Recipe 6=0% red: 100% blue; CK: Fluorescent lamp.*

Lettuce showed a stressed response against LED treatment as well; recipe 1 refers to the monochromatic red light that had strongly reduced fresh matter to 64% compared with recipe 3. However, recipe 3 revealed a positive effect on biomass compared to CK, the dry matter was increased by 26% while no significant difference in the fresh matter was observed. As a consequence, this fact beneficially resulting in accumulation and water use efficiency. The monochromatic blue light was extremely positive associated with lettuce height, which increased around 15%~19% instead of other treatments. Yet, in terms of the leaf area, a negative response was fund, indicating the evitable role that blue light is playing in the stem elongation. Generally speaking, lettuce showed a similar tendency compared with tomato, the accumulation was stressed at different levels by LED recipes. Only, the blue light had the opposite effect on stem elongation, as the tomato height was inhibited by increasing blue proportion. Whereas the 100% blue light promoted the stem height of lettuce.

Table 4. 1. 3 *The post-harvested pak choi physiological features when grew under different red/blue ratio for 30days*

Treatment (Recipes)	Plant height (mm)	Fresh matter (g)	Dry matter (g)	Leaf area (cm2)
CK	5.24±0.05d	13.421±0.0.043e	1.047±0.075c	26.154 ±1.052c
Recipe 1	6.61±0.06b	15.290±0.062d	0.980±0.041c	26.516 ±0.768c
Recipe 2	6.72±0.05b	15.784±0.088d	1.117±0.041ab	27.453 ±0.221b
Recipe 3	6.74±0.15b	15.691±0.030d	1.237±0.026b	29.840 ±0.598a
Recipe 4	6.17±0,10c	19.077±0.403a	1.434±0.009a	27.565± 0.431b
Recipe 5	6.12±0.04c	16.884±0.706c	1.217±0.013a	27.951 ±0.521b
Recipe 6	7.21±0.08a	18.328±0.684b	1.008±0.027c	24.537 ±0.831d

**Within a column, means followed by different letters are significantly different at P ≤0.05, n=3; values after ± are standard deviation values. Recipe1=100% red: 0%blue; Recipe 2=77% red: 23% blue; Recipe 3=73% red: 27% blue; Recipe 4=70% red: 30% blue; Recipe 5=62% red: 38% blue; Recipe 6=0% red: 100% blue; CK: Fluorescent lamp.*

Compared with CK, the LED treatment benefit the pak choi yield significantly ($P<0.05$), as well as the plant's other respective, including height, fresh matter, and dry matter. Unlike other species, pak choi demonstrated a vigorous response to recipe 4, the fresh matter augmented by 42% compared with CK, and 13%~24% increase compared with other LED recipes. It had been observed that blue light proportion increased from 27% to 30%, accompanied by the fresh matter advent to 21%, and there was no significant difference once blue light changed to 100%. However, the dry matter of recipe 3 was 23% higher than recipe 6, indicating that monochromatic blue light helped generating the water content in leafy vegetables; relatively the monochromatic red light works on promoting leaf expanding. In generally consideration, pak choi showed

positive reaction to LED treatments, particularly recipe 4 that conduced prominent ($P<0.05$) accumulation improvement and leaf expansion. The monochromatic blue light showed negative phenotypic effect in fresh matter and dry matter compared with recipe 4 but exhibited conspicuous elongation in stem and hypocotyl leading to spindling growth when treated by recipe 6 (Figure 4. 1. 2).

Figure 4. 1. 2 *The extremely extended petiole of pak choi (from 13mm to 42mm) under 100% blue light treatments. From left to right the treatments refer to 27%, 30%, 38% and 100% blue light*

It has also been noticed that with additional far-red light, the pak choi flowering time can be extremely brought forward. During the experiment, the pak choi started flowering at 20 days with added far red light and others were not flowering for all growth periods (Figure 4. 1. 3).

Figure 4. 1. 3 *Pre-harvest pak choi growth status. From left to right are fluorescent treatment, recipe 1 (red/blue: 90%/10%), recipe 2 (red/blue: 70%/30%) and recipe 3 (red/blue: 70%/30% with added far red). The recipe 3 treatments started flowering after 20 days growth. Image shows the growth status at 30th day.*

4. 1. 2 Changed intensity on yield and energy consumption.

Table 4. 1. 4 presents the results of how different light intensity influence pak choi growth. As the plants were growing under the same LED recipes (red/blue at 70%/30%) but different light intensity (recipe L, M and H refers to 100, 150, and 200 μmol/m^2s, respectively), the measurements indicated that the fresh matter was raised as the intensity increasing, but recipe M had the highest chlorophyll content among LED recipes. Fluorescent lamp treatment (CK) had

the highest yield and chlorophyll content and had the highest energy consumption. With the same intensity, CK showed 16% higher yield than recipe H, indicating that pak choi was stressed with 200 μmol/m^{2}s LED lighting.

Table 4. 1. 4 *After 60 days of growth the pak choi physiological features and the total energy cost when grew under different light intensity but same red/blue ratio, compared with fluorescent treatment as control (CK).*

Treatment	Fresh matter (g)	Dry matter (g)	Chlorophyll content (mg/g)	Energy consumption (Kwh)
CK	71.96±4.701a	3.95±0.294a	2.245±0.101a	108
Recipe H	61.66±3.270b	2.86±0.332b	1.547±0.012c	43.2
Recipe M	41.367±1.995c	2.11±0.210c	1.825±0.031b	28.8
Recipe L	19.893±1.334d	1.20±0.095d	1.132±0.025d	19.2

**Within a column, means followed by different letters are significantly different at P ≤0.05, n=3. Recipe H, Recipe M and Recipe L stands for high intensity treatment (200μmol/m^{2}s), medium intensity treatment (150μmol/m^{2}s) and low intensity treatment (100μmol/ m^{2}s), respectively.*

Although CK had significantly improved biomass and chlorophyll content, its extremely higher total energy cost it presented inefficient energy usage. On the other hand, LED treatments showed positive relationships between biomass and energy cost. As each LED panel only cost 40% energy compared with CK, the Energy Use Efficiency (EUE) was 114% higher than CK (Figure 4. 1. 4). The EUE between recipe H and M showed no significant difference, indicating similar Energy-Light-Biomass conversion efficiency. Considered as 150 μmol/m^{2}s will be suitable for industrialized targets, recipe M has the most potential to be extensively applied for actual industrial production and marketing

management.

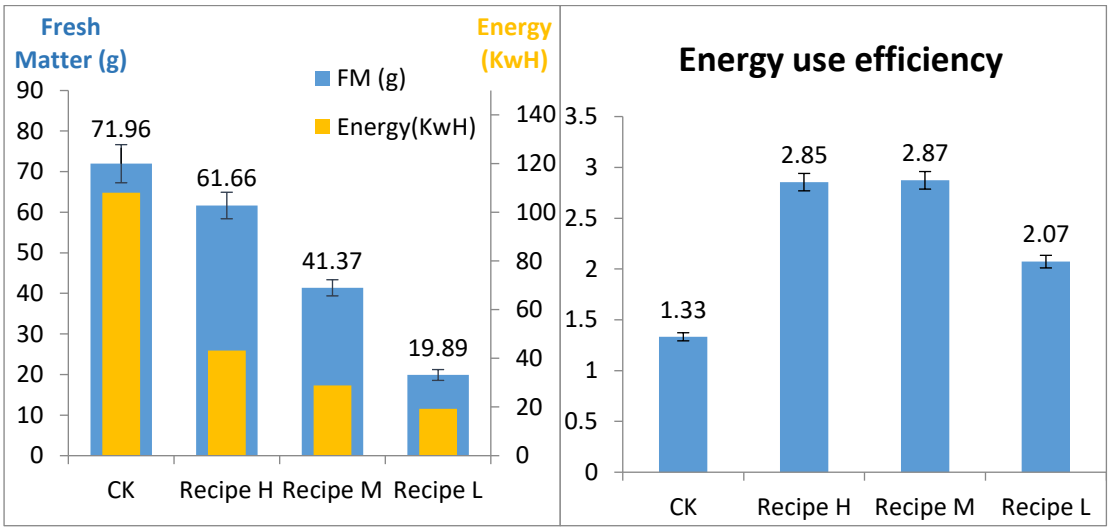
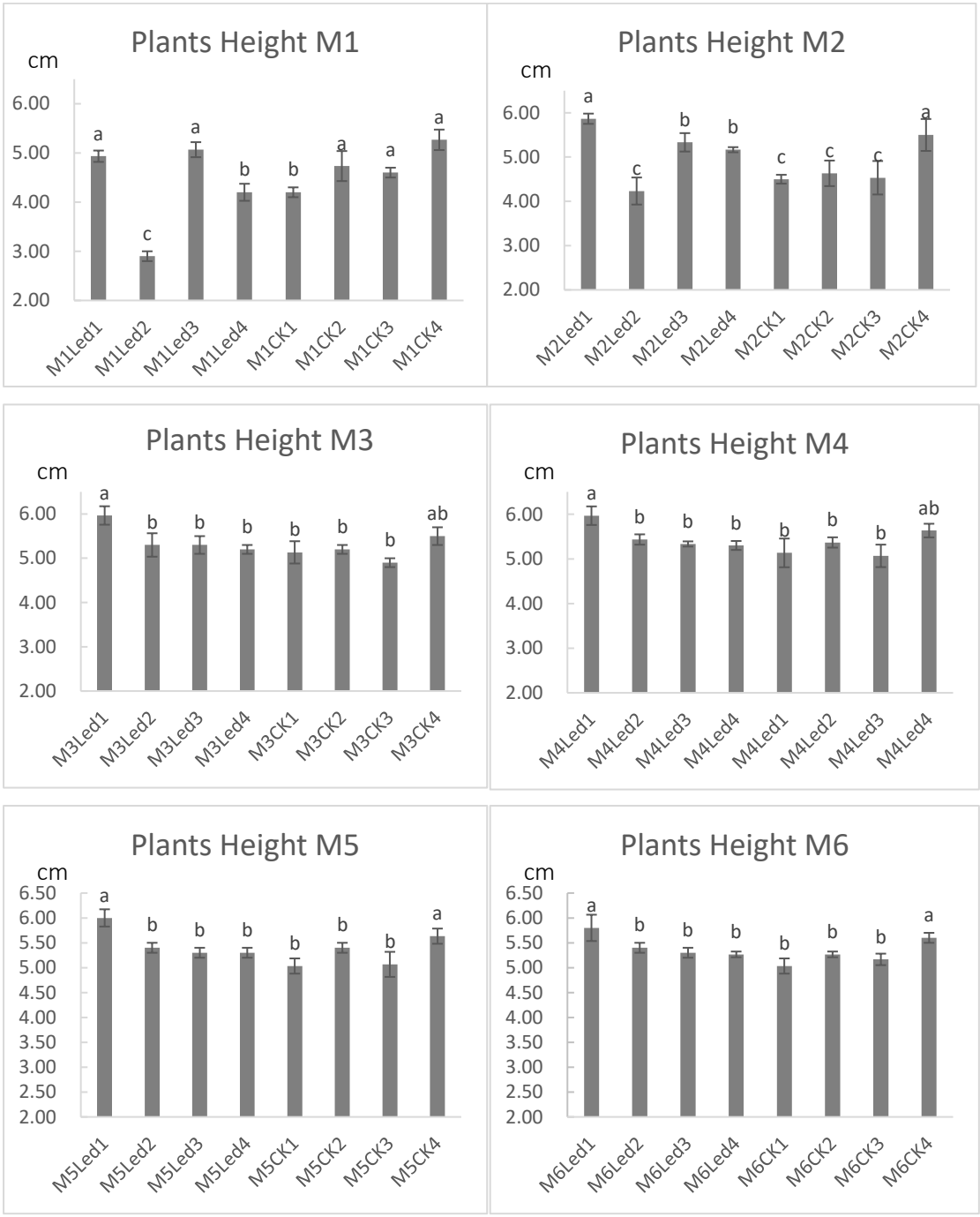


Figure 4. 1. 4 *Energy use efficiency (Fresh matter/total energy cost) between different LED recipes. Energy presented the total electric used during 60 days growth. Means followed by different letters are significantly different at P ≤0.05, n=3.*

3.3.3 Effects of LED recipes on four pak choi varieties growth

The results of growth status between four varieties of pak choi have been shown below.


Plants Height M1
cm
6.00
5.00
4.00
3.00
2.00
a
c
a
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b
a
a
a
M1Led1
M1Led2
M1Led3
M1Led4
M1CK1
M1CK2
M1CK3
M1CK4
Plants Height M2
cm
6.00
5.00
4.00
3.00
2.00
a
c
b
b
c
c
c
a
M2Led1
M2Led2
M2Led3
M2Led4
M2CK1
M2CK2
M2CK3
M2CK4
Plants Height M3
cm
6.00
5.00
4.00
3.00
2.00
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b
b
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b
b
b
ab
M3Led1
M3Led2
M3Led3
M3Led4
M3CK1
M3CK2
M3CK3
M3CK4
Plants Height M4
cm
6.00
5.00
4.00
3.00
2.00
a
b
b
b
b
b
b
ab
M4Led1
M4Led2
M4Led3
M4Led4
M4Led1
M4Led2
M4Led3
M4Led4
Plants Height M5
cm
6.50
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5.00
4.50
4.00
3.50
3.00
2.50
2.00
a
b
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M5Led1
M5Led2
M5Led3
M5Led4
M5CK1
M5CK2
M5CK3
M5CK4
Plants Height M6
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M6CK1
M6CK2
M6CK3
M6CK4

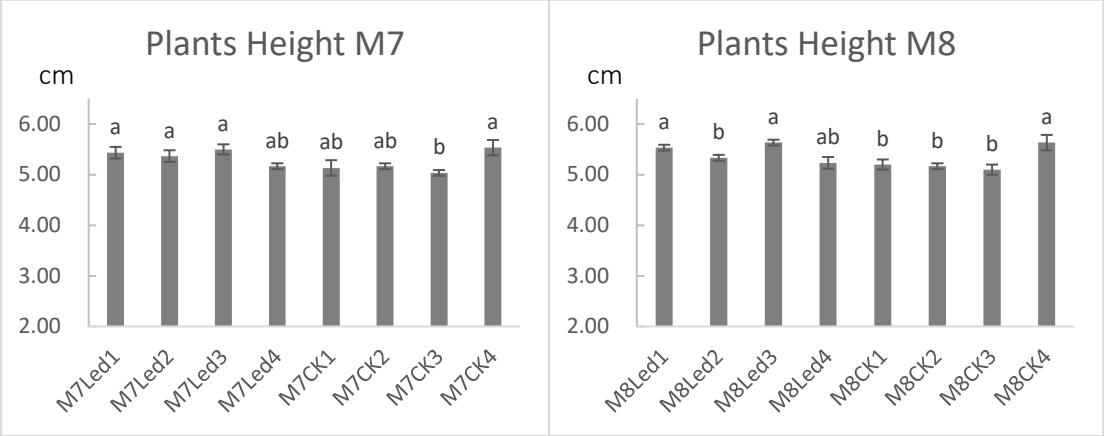
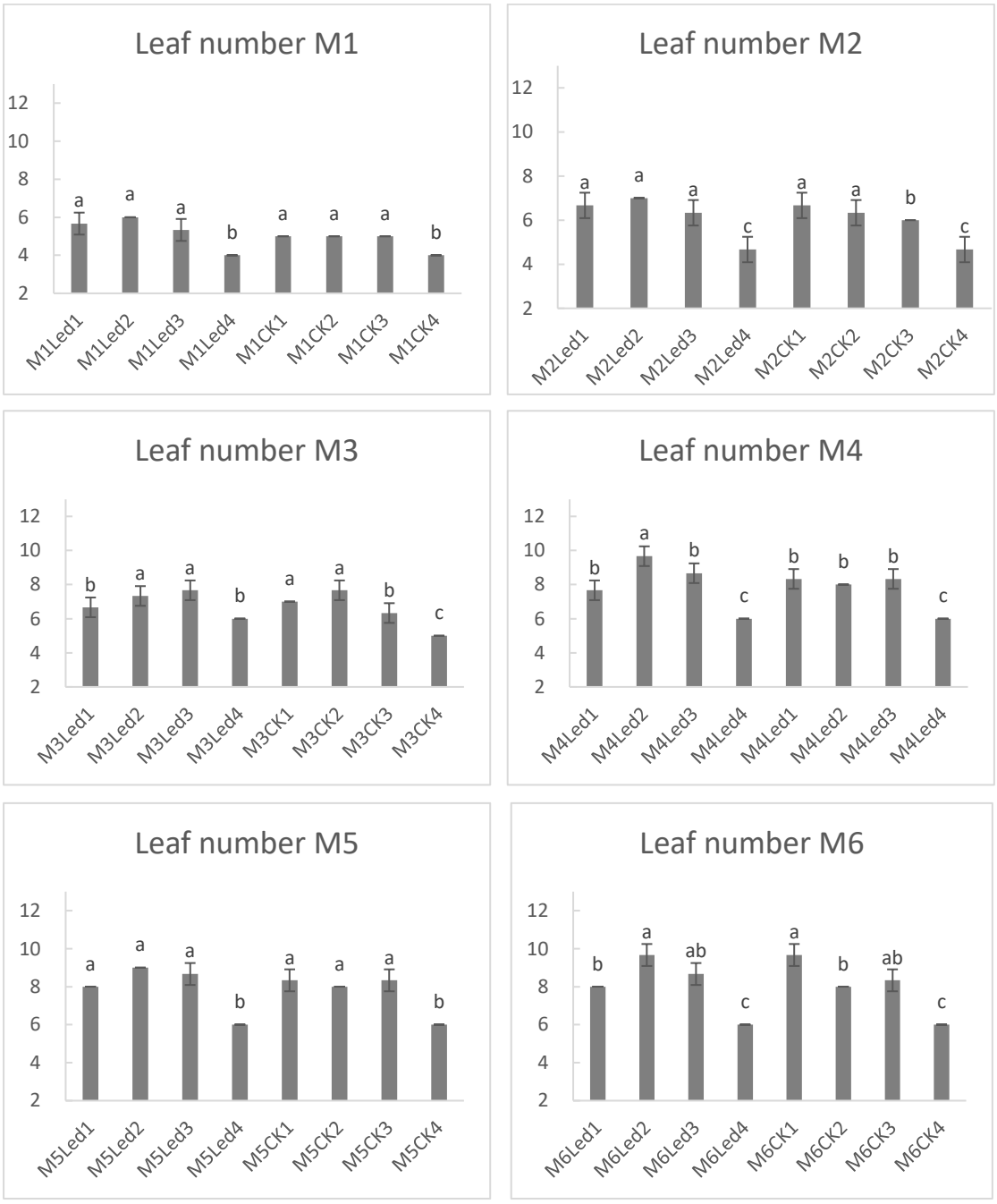


Figure 4. 1. 5 *Plant height measurements during growth. Led 1 refers to 802 Bushido; Led 2 refers to Kungfu 2; Led 3 refers to 702 Warrior, Led 4 refers to Green Hill 402, treated by red/blue 7/3 LED light. CK 1, 2, 3 and 4 refers to the same varieties but treated by fluorescent lamp as control. M1 stands for the first measurement at 3rd day, M2 stands for the second measurement at 6th day, followed by this pattern. The labelled different letters are significantly different at P ≤0.05, n=3.*

The Kungfu 2 (Led 2) showed a significantly ($P<0.05$) different response between LED treatment and CK. During the first two measurements (M1 and M2), the plant height of Led 2 was depressing compared with fluorescent lamp treatment (CK 2), and it was also the lowest reading among all other treatments. Green Hill 402 treated by LED light (Led 4) was also repressed in plant height as CK4 was promoted. 802 Bushido (Led 1) and 702 Warrior (Led 3) extended most quickly in M1 and M2. However, 802 Bushido treated by the fluorescent lamp (CK 1) had the lowest reading among control. The dissimilarity in M1 and M2 showed a significant difference in earlier stage growth speed of four varieties, but the maximum height of all treatments was around 5.5 cm. From M6, there was no significant ($P>0.05$) difference between all treatments. Although Led 2 was genuinely stressed at the earlier stage but it reached the peak at M4, showed the most rapid growth speed. Led 1 was most adaptable

to the LED treatment, which maintained the highest point through all measurements (Figure 4. 1. 5).



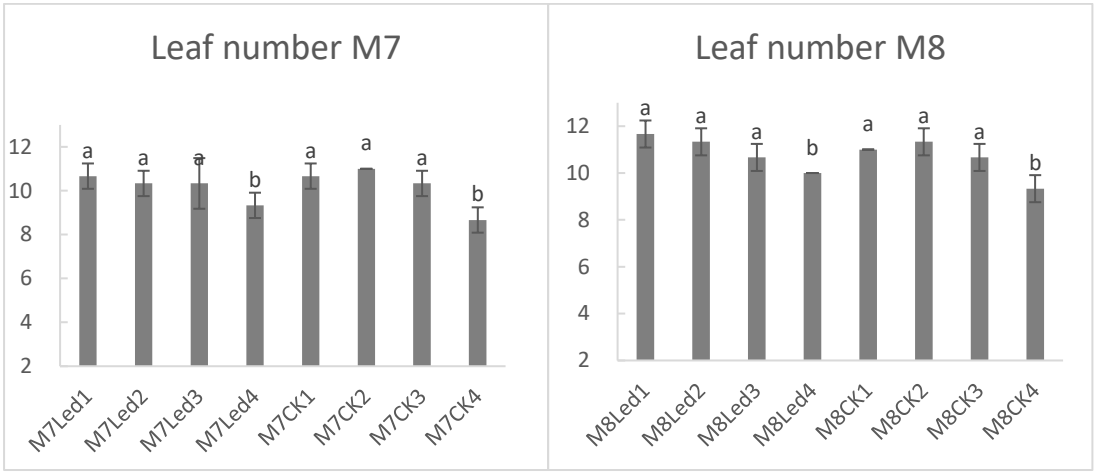
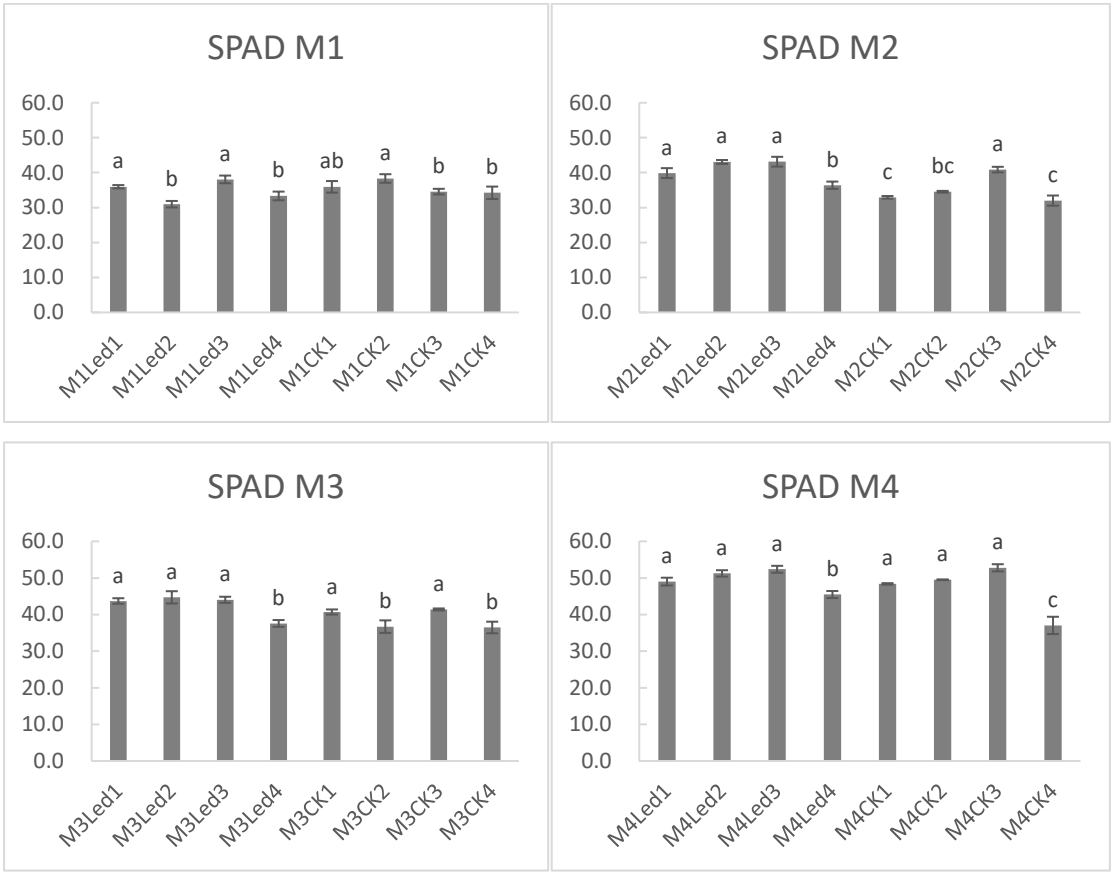


Figure 4. 1. 6 *Leaf number measurements during growth from 3rd day after transplanting. The leaf which was fully expanded from shoot can be calculated. Led 1 refers to 802 Bushido; Led 2 refers to Kungfu 2; Led 3 refers to 702 Warrior, Led 4 refers to Green Hill 402, treated by red/blue 7/3 LED light. CK 1, 2, 3 and 4 refers to the same varieties but treated by fluorescent lamp as control. M1 stands for the first measurement at 3rd day, M2 stands for the second measurement at 6th day, followed by this pattern. The labelled different letters are significantly different at P ≤0.05, n=3.*

Unlike the observation in plant height measurements, Led 2 showed the most positive response against LED light treatment in leaf number, which had the highest number through all growth stages ($P<0.05$). It was clear to observe an accelerated increase in Led 2 from M4 to M6, yet all varieties had similar results of maximum leaf number, except Green Hill 402, which was presented decreasing reads in Led 4 and CK 4 ($P<0.05$). The leaf number of 804 Bushido was suppressed with LED treatment (Led 1), which designate a different response with its plant height (Figure 4. 1. 6).

For SPAD results, the differences between LED and fluorescent treatments were not significant ($P>0.05$). 802 Bushido (Led 1) had higher chlorophyll content in M2, but no variance in other treatments and the content was even

decreased 12% in M8 (P<0.05). Green Hill 402 (Led 4) and Green Hill 402 (CK 4) had no difference before M3, but the chlorophyll appeared to be limited afterwards. In M5, the CK 4 was 19% higher than Led 4 (P<0.05). As CK 4 presented the lowest reading among all other controls, in the same way, Led 4 also had a limited amount compared with other LED treatments, but at the final stages, all treatments reached the equivalent level, see details in Figure 4. 1. 7.



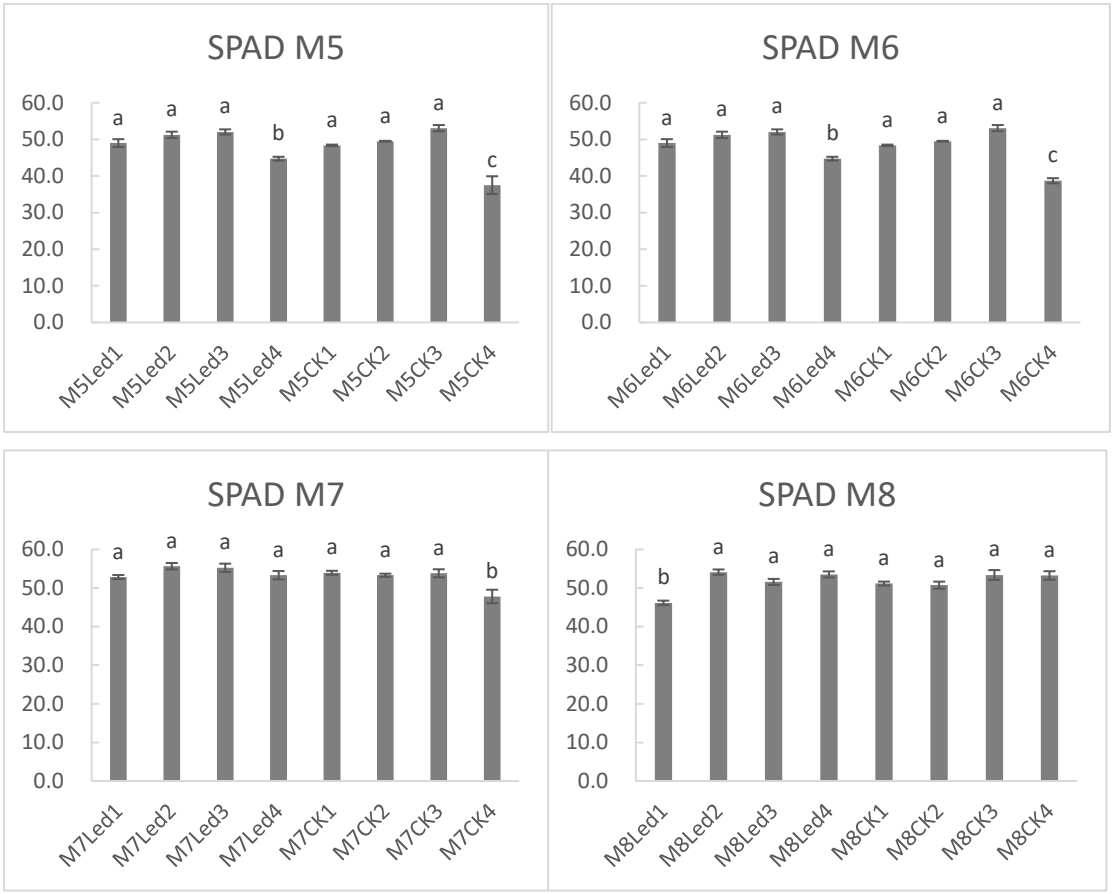
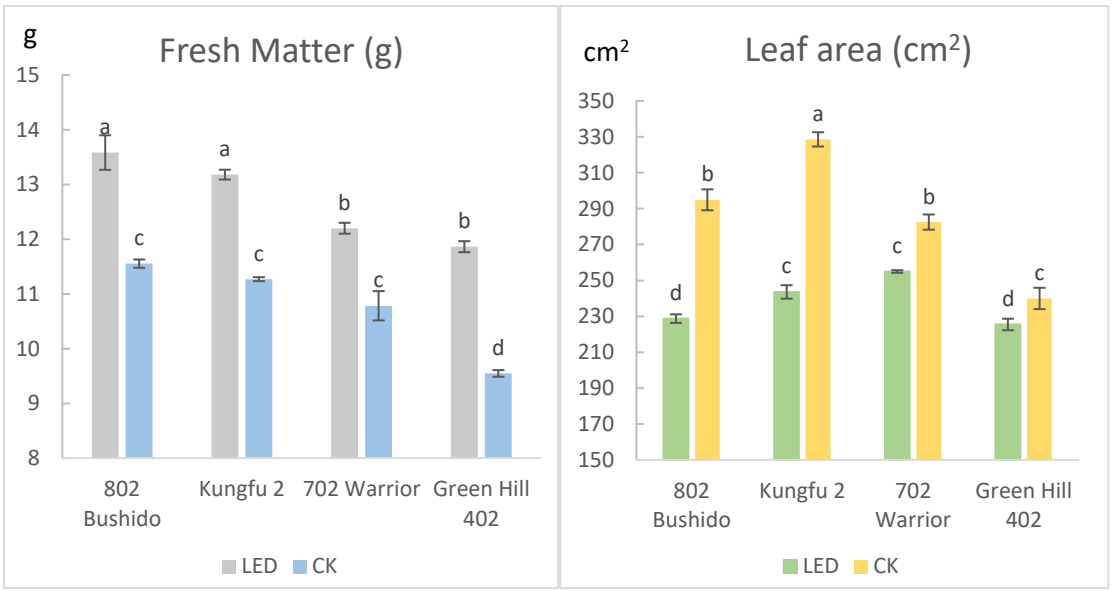
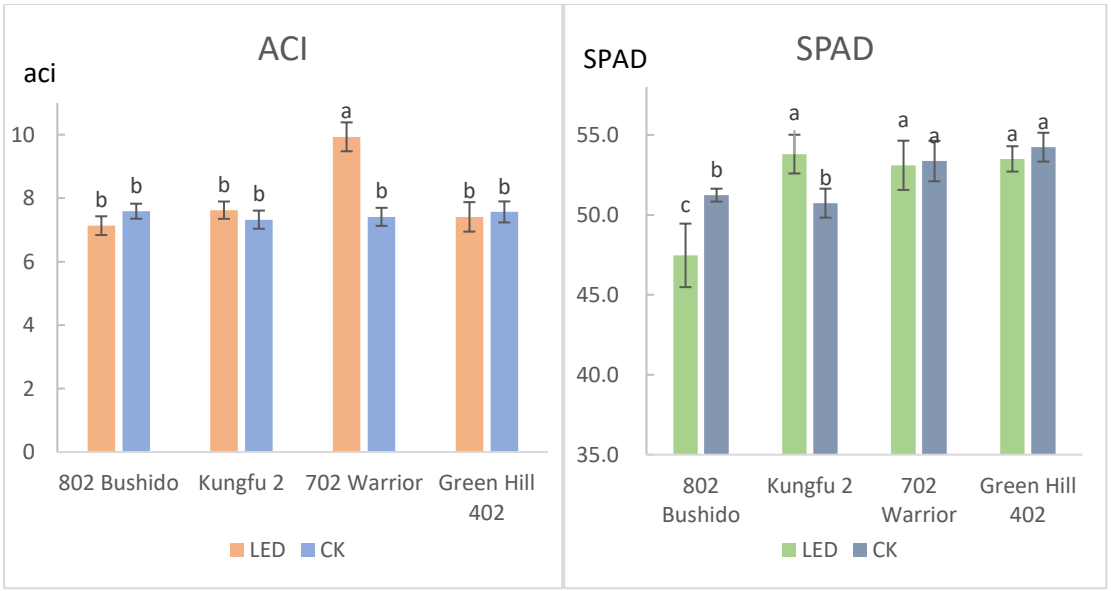


Figure 4. 1. 7 *SPAD reading of four pak choi varieties during growth. The measurements were proceeded on the third fully expanded leaf. Led 1 refers to 802 Bushido; Led 2 refers to Kungfu 2; Led 3 refers to 702 Warrior, Led 4 refers to Green Hill 402, treated by red/blue 7/3 LED light. CK 1, 2, 3 and 4 refers to the same varieties but treated by fluorescent lamp as control. M1 stands for the first measurement at 3rd day, M2 stands for the second measurement at 6th day, followed by this pattern. The labelled different letters are significantly different at P ≤0.05, n=3.*

In General, the plant height, leaf number and SPAD reads of the four varieties had similar maximum value. After 30 days of growth, the reads of them had no significant difference. However, at the earlier growth stage, they showed dramatically variant responses. The variety with higher growth speed in plant

height typically showed inhibited features in leaf number and chlorophyll content, symbolising a competitive relationship between stem elongation and leaf development.





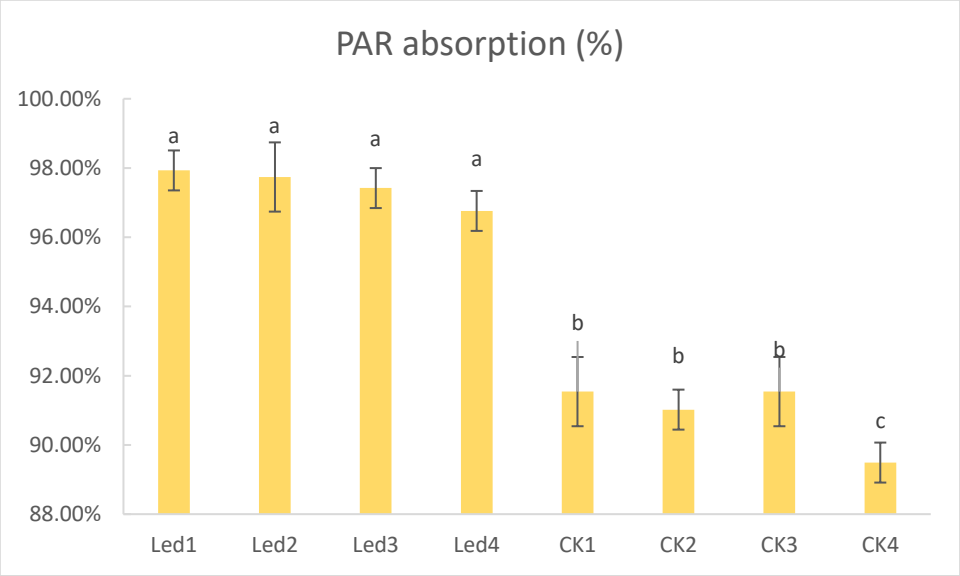


Figure 4. 1. 8 *Pre-harvested pak choi physiological features. After 30 days growth the sample was first measured by SPAD, PARu and ACI, then the above-ground part was harvested and weighed, showed as fresh matter; after weigh the leaves were all removed from stem and measured for leaf area. PAR absorption was calculated as (PAR-PARu)/PAR. The labelled different letters are significantly different at P ≤0.05, n=3.*

Although the diversity of morphology has been observed between different pak choi varieties and LED treatments, the fresh matter was increased by 17.58% in 802 Bushido, 16.95% in Kungfu 2, 13.17% in 702 Warrior, and 24.19% in Green Hill 402, respectively compared with CK. 802 Bushido had the highest biomass accumulation among four varieties, and the yield of Green Hill 402 was not satisfied, especially when treated by a fluorescent lamp. The yield was decreased by at least 12.94% compared with other CK ($P<0.05$). In contrast, the total leaf area revealed different results. All varieties showed negative results when treated by LED recipes compare with CK. It was increased by 28.95% in 802 Bushido, 34.9% in Kungfu 2, 10.79% in 702 Warrior, and 6.44% in Green Hill 402, respectively, compared with CK ($P<0.05$). Kungfu 2 showed

the most negative response in the leaf area with LED treatment. When treated by the fluorescent lamp, it acquired the highest amount between four varieties. 802 Bushido had the lowest result with LED treatment as it was 10.29% decreased compared with 702 Warrior, which had the highest value in LED treatments ($P<0.05$). Although these four kinds of pak choi showed improved leaf area under fluorescent lamp, the PAR absorption indicated significant different results. The average PAR absorption in LED treatment was 97.46%, and the result in CK was just 90.90%, which was increased to 107.22% by LED lighting ($P<0.05$). The reads between varieties showed no significant difference, expect Green Hill 402 was the lowest in CK. On the other side, the chlorophyll content was measured before harvested by SPAD; it presented that 802 Bushido was decreased by 7.38% in LED treatment, though Kungfu 2 was increased by 6.11% in an LED recipe ($P<0.05$). 702 Warrior and Green Hill 402 showed no difference in chlorophyll content. For anthocyanin content (ACI), 702 Warrior showed an immensely enhanced result with LED treatment; the ACI read was increased by 34.00% compared with CK, which also reached the peak of all LED treatment ($P<0.05$), but its CK had no difference with other varieties. The ACI of 802 Bushido, Kungfu 2 and Green Hill 402 explicated similar results with LED treatment. In general, these four varieties still presented significant diversity in post-harvested results. Although the yield was enhanced regularly by LED treatments, the leaf development was very variety-specific. Take the during-growth measurements into consideration; it also indicated that as 802 Bushido had the highest amount in biomass after 30 days of growth, the SPAD and leaf area explicated decreasing tendency, this may reveal that the production capacity still changing at later stage and proffered us new insights on the relationship between varieties and light conditions (Figure 4. 1. 8).

In Table 4. 1. 5, it is quickly observing that Kungfu 2 with LED treatment showed remarkable improvement in root structure, the total length of the root was increased by 20.96% approximately, while the total surface area improved by

23.95%. There were no significant changes in average diameter, but it still promoted total root volume. Green Hill 402 was extremely repressed in total root length, which is only 51.87% of the reading in Kungfu 2, but in contrast, the average diameter was 21.6% higher than other varieties, which still presented decent results in total root volume, and it had the lowest amount in tips number. 702 Warrior had similar reading in total root length with 802 Bushido, but it had a 13.5% increased diameter and presented a similar result in total root volume, compare with Kungfu 2. Meanwhile, 802 Bushido performed regularly in each measurement.

However, the root development with fluorescent treatment showed inconsistent results. The total root length from 802 Bushido was remarkably inhibited, the reading was reduced to 73.18% compared with Green Hill 402. In contrast the average diameter of 802 Bushido was significantly enhanced to 155%, which leaded to 34.96% more total root volume than Green Hill 402, but it still had the lowest readings in total tips number. Unlike the performance in LED treatment, Kungfu 2 had unsatisfied results with fluorescent lamp treatments. With an average level in total root length, Kungfu 2 appeared to acquire nearly the lowest reading in diameter, which presented 34.66% decreased total root volume and 19.06% reduced in total surface area, compared with 702 Warrior. 702 Warrior highly performed in root volume, average diameter, and total surface area, which showed the most emphatic response to fluorescent treatment. Green Hill 402 had well-developed root length with fluorescent treatment; however, due to the limitation in average diameter, it presented the lowest total root volume and surface area, yet the highest reading in tips number, which showed a 32.17% increased than 702 Warrior (Table 4. 1. 5).

Table 4. 1. 5 *Post harvested pak choi root scanning. The analysis including total root length (Length); total root surface area (Surf Area); average diameter of total root hairs; total number of root tips (Tips), total number of root forks (Forks); and total number of root crossing (Crossing).*

LED treatments	Length(cm)	Surf Area (cm^2)	Avg Diam (mm)	Root vol(cm^3)	Tips	Forks	Crossing
802 Bushido	2695.42±42.50c	309.01±15.07c	0.37±0.01b	2.96±0.02b	9698.67±656.54a	27684±2151.06b	6944±754.21b
Kungfu 2	3260.22±68.84a	383.13±9.33a	0.37±0.01b	3.79±0.06a	10742.67±2924.02a	32486±2107.37a	7910±572.44a
702 Warrior	2904.20±47.83b	340.63±2.23b	0.42±0.01a	3.62±0.09a	8308±1752.17a	29945.33±2547.10b	6348.67±420.35b
Green Hill 402	1691.53±98.70d	225.67±17.16d	0.45±0.02a	2.67±0.08c	5992±604.87b	20761.33±1927.01c	4500.67±243.54c
Fluorescent light treatments (CK)	**Length(cm)**	**Surf Area (cm^2)**	**Avg Diam (mm)**	**Root vol(cm^3)**	**Tips**	**Forks**	**Crossing**
802 Bushido	1711.01±337.57b	245.39±5.98c	0.51±0.01a	3.05±0.07b	5317.33±180.01c	21145.33±149.36b	4363±30.20c
Kungfu 2	2344.18±181.18a	290.72±9.85b	0.39±0.01c	2.81±0.14c	7850.00±433.73b	25312±264.48b	6107±195.59a
702 Warrior	2492.58±149.36a	359.19±2.77a	0.46±0.03b	4.30±0.40a	7304.67±248.90b	30038±1340.58a	6365±331.92a
Green Hill 402	2338.85±216.92a	256.91±13.74c	0.33±0.02d	2.26±0.06d	9654.00±391.76a	24431.33±1037.59b	5409±363.33bb

**Within a column, means followed by different letters are significantly different at P ≤0.05, n=3.*

In conclusion, 802 Bushido was slightly inhibited with LED treatment, and especially the diameter was lower than others. The length was further reduced with fluorescent treatment, but the diameter was contrarily improved, leading to similar results in total root volume. Kungfu 2 was significant enhanced in root length with LED treatment to obtain the highest root volume, but with fluorescent treatment, the diameter was reduced, leading to relatively lower root volume and surface area. 702 Warrior showed impressive enhancement in both LED and fluorescent treatments, especially the total root volume reached the peak in fluorescent treatment compared with all other samples. However, Green Hill 402 performed unsatisfying in both treatments, even though it had the highest diameter under the LED light. Typical root scan images of four varieties have are showing in Figures 4. 1. 9 to 4. 1. 12.

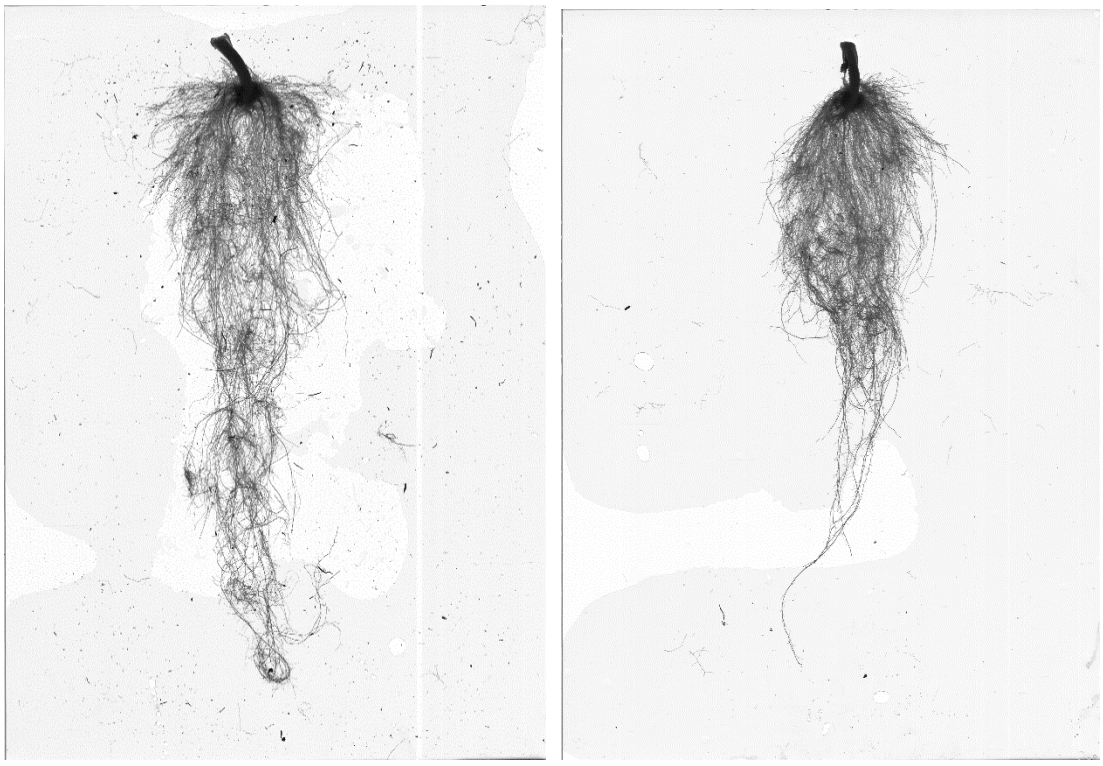


Figure 4. 1. 9 Root image for 802 Bushido with LED treatment (left) and CK (right)

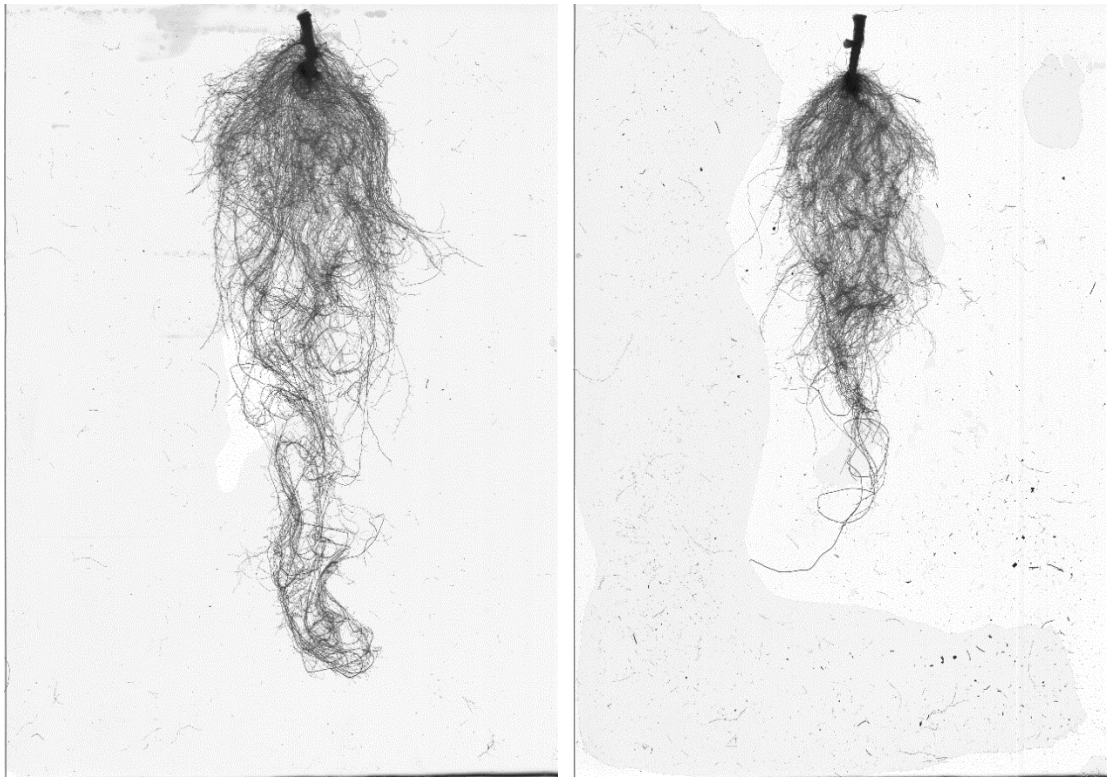


Figure 4. 1. 10 Root image for Kungfu 2, with LED treatment (left) and CK (right)

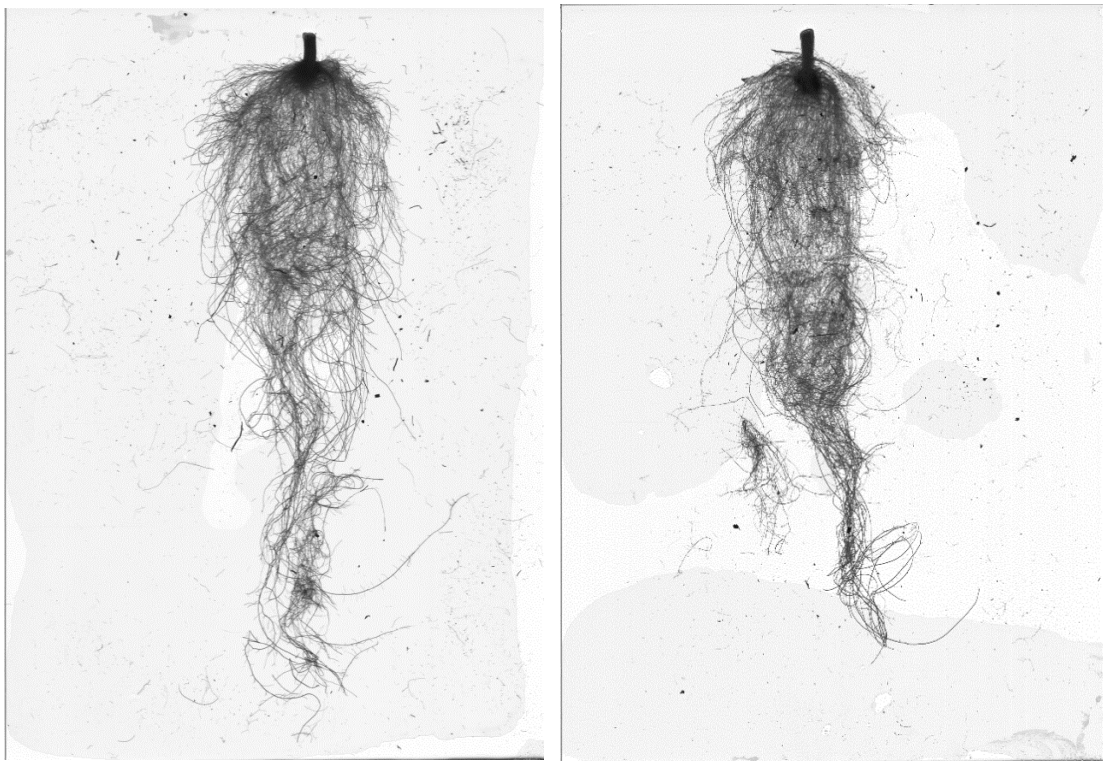


Figure 4. 1. 11 Root image for 702 Warrior, with LED treatment (left) and CK (right)

Figure 4. 1. 12 Root image for Green Hill 402, with LED treatments (left) and Fluorescent light treatments (right)

4. 2. Effects of LED Lighting on Photosynthesis

In Section 4.1, the diverse and complex physiological responses between different subjects caused by changeable LED recipes have been identified, and the detailed comparison was carried out within yield, morphological characterisation, leaf development and root structure. As photosynthesis progress is directly related to the lighting spectrum, to further understand the regulation from different LED recipes, this section chose pak choi as the main subject to explore the effects of LED recipes on photosynthesis mechanism by using gas exchange and chlorophyll fluorescence kinetics technique. Of particular engagement was (1) the tolerance to light stress, (2) the impact of changes in LED recipes on the light utility efficiency and (3) the differential response between varieties.

4. 2. 1 Effects of LED recipes on light curve

Pak choi was grown under three LED recipes: Recipe M (red/blue: 70%/30%); Recipe R (100% red) and Recipe B (100% blue). After 30 days of growth before harvested, the samples were measured by LiCor light curve, compared with fluorescent treatment as control (CK).

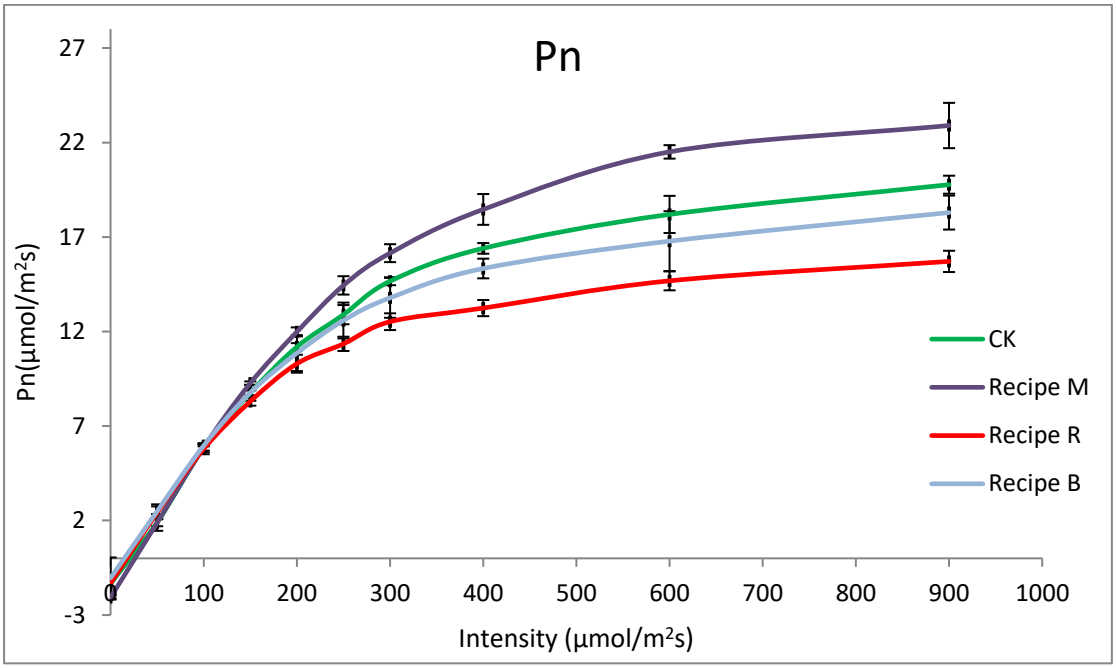


Figure 4. 2. 1 *The light curve of Net photosynthetic rate (Pn) of pak choi treated with different LED recipes: Recipe M (red/blue: 70%/30%); Recipe R (100% red) and Recipe B (100% blue). The error bars are standard deviation values (n=3).*

Figure 4. 2. 1 for net photosynthetic rate measurement shows that when light intensity was at 0 μmol/m^{2}s, Recipe M demonstrated the lowest reading, which refers to the highest photorespiration. As the intensity increased to 100 μmol/m^{2}s, there was no significant difference observed between each treatment ($P > 0.05$), which indicated that the prominent inhibition was the lack of photon flux. After that, Recipe M remained the highest reading in the light curve, followed by CK, Recipe R and Recipe B, respectively. There was a clear boost for CK at 250μmol/m^{2}s, and pak choi treated with a relatively more completed spectrum showed better tolerance to higher light intensity than monochromatic light, but still lower than combined red/blue at 70 % / 30 % recipe.

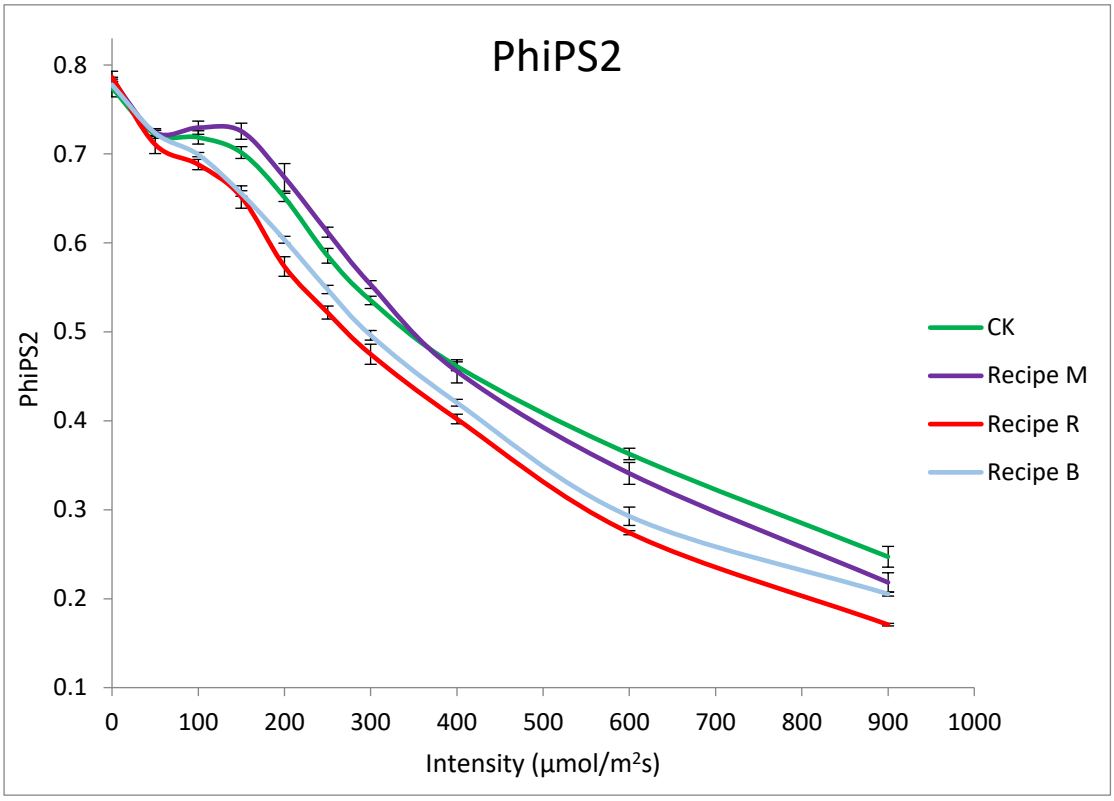


Figure 4. 2. 2 *Light curve of actual photochemical efficiency of PS II in the light in pak choi treated with LED recipes: Recipe M (red/blue: 70%/30%); Recipe R (100% red) and Recipe B (100% blue). The error bars are standard deviation values (n=3).*

Meanwhile, the actual photochemical efficiency of the PS II center without dark-adapted was measured from different LED treatments (Figure 4. 2. 2). In the intensity range which generally used for production (100-200µmol/m^{2}s), Recipe M with 70% red and 30% blue spectrum showed a positive response in PS II reaction center, followed by CK, Recipe B and Recipe R, which was the same tendency as the previous experimental results. However, after light intensity raised to 350µmol/m^{2}s, Recipe M was overtaken by CK and remained after. Recipe R and Recipe B had no significant difference at 150 µmol/m^{2}s, except that Recipe B performed better than Recipe R during all light intensity. It may betoken that blue light has positively affected pak choi PS II light stress tolerance, but combined spectrum such as Recipe M still performed better

during specific intensity range, which is also suitable for commercial production. Above that, fluorescent lamp treatment showed better tolerance compared to all LED recipes.

The stomatal conductance measurement (Figure 4. 2. 3) showed the curves of the stomatal movement against increasing PAR. LED recipe with 100% blue light (Recipe B) had a great promotion to stomatal movement, which maintained the highest point through all light curve measurements, followed by Recipe M, CK and Recipe R. After light intensity reached 300μmol/m^{2}s, Recipe M and CK had no significant differences ($P > 0.05$). Compared with other measurements, Cond in each treatment had no significant changes after 300μmol/m^{2}s, indicating that the intensity became the limiting factor rather than the lighting spectrum for stomatal movement.

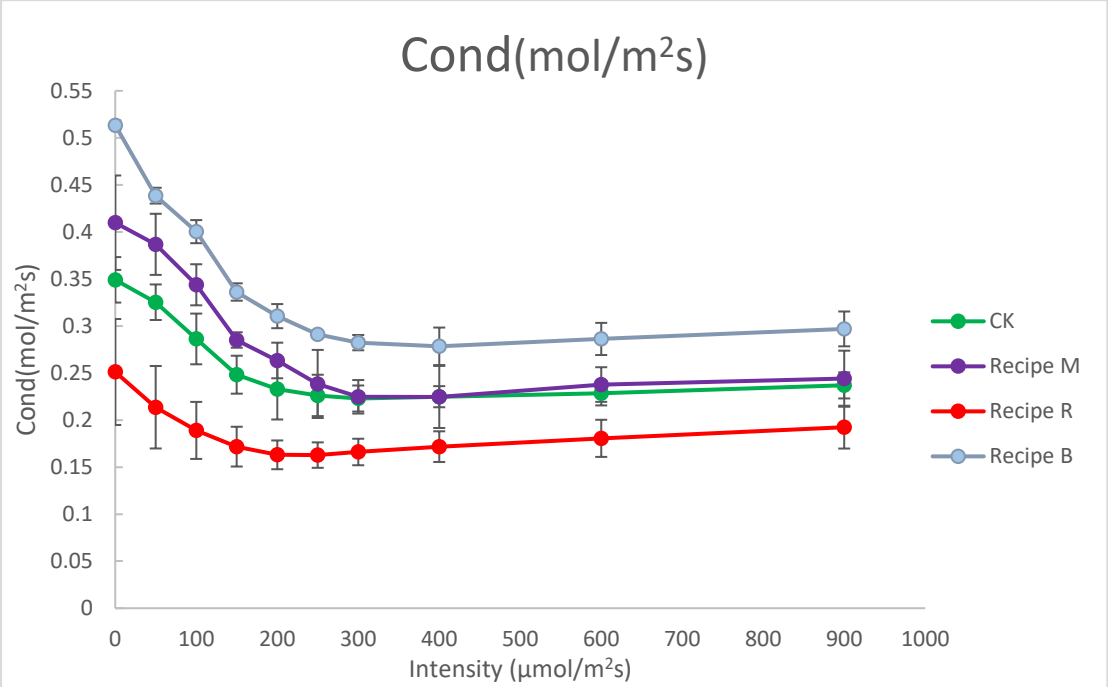


Figure 4. 2. 3 *The stomatal conductance (Cond) of pak choi leave under different LED recipes. Recipe M (red/blue: 70%/30%); Recipe R (100% red) and Recipe B (100% blue). The error bars are standard deviation values (n=3).*

4. 2. 2. Effects of different LED recipes on pak choi photosynthesis

Pak choi (*Brassica rapa. Green revolution F1*) growth in UK campus was treated with six different LED recipes, the growth conditions and detailed LED recipes spectrum has been showing in section 3. 4 to 3. 5.

The pak choi were grown with changed LED recipes: Recipe 1 (red: blue 100%/0%); Recipe 2 (red: blue 77%/23%); Recipe 3 (red: blue 73%/27%); Recipe 4 (red: blue 70%/30%); Recipe 5 (red: blue 62%/38%); and Recipe 6 (red: blue 0%/100%), all under 130μmol/m^2s intensity, compared with fluorescent lamp treatment as CK. The Pn and PhiPS2 showed a similar tendency, as Recipe 1 all had the lowest performance, meanwhile recipe 6 had the lowest reading in Pn but relatively higher in PhiPS2, which indicated that monochromic red light was strongly stressing PS II center, yet monochromic blue light can still meet its requirement. Recipe 4 demonstrated a significantly higher level in Pn than other treatment, which showcased as the highest carbon assimilation rate. In contrast, Recipe 3 recorded higher performance in PhiPS2, while Recipe 4, 5 and 6 had no significant difference, which suggested that the PS II reaction center required certain content of blue light to maintain its function, more like a regulation factor rather than photochemistry resource. Fv/Fm as another evidence showed that recipe 6 had the highest volume even compared with CK, while CK had no difference with Recipe 3, 4, and 5, which also proved that blue light could enhance the stress tolerance in PSII center, and Recipe 1 (monochromic red light) still showed a negative effect on PSII center. Meantime, the photochemical quenching treatment exposed the equivalent tendency with PhiPS2 results, except Recipe 3 remained the same level as Recipe 4, 5 and 6 (Figure 4. 2. 4).

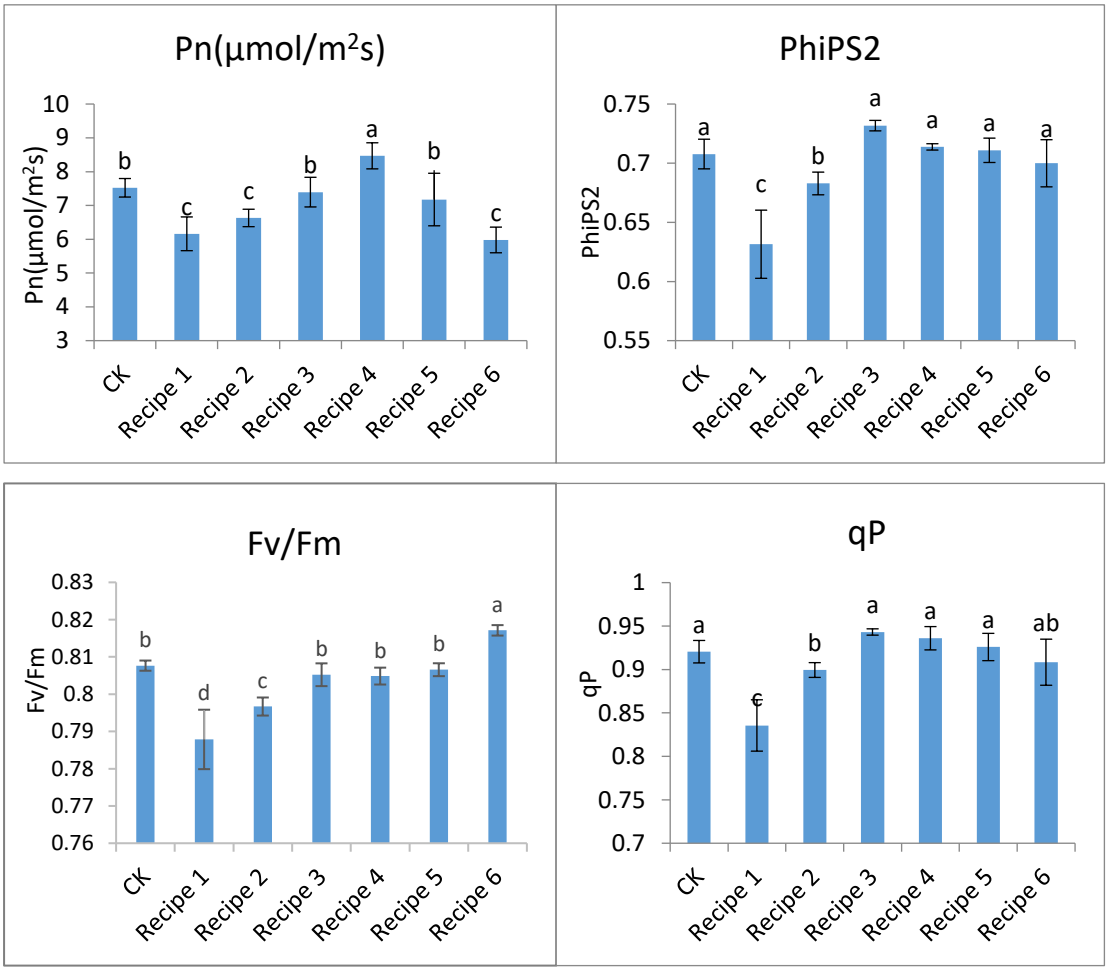
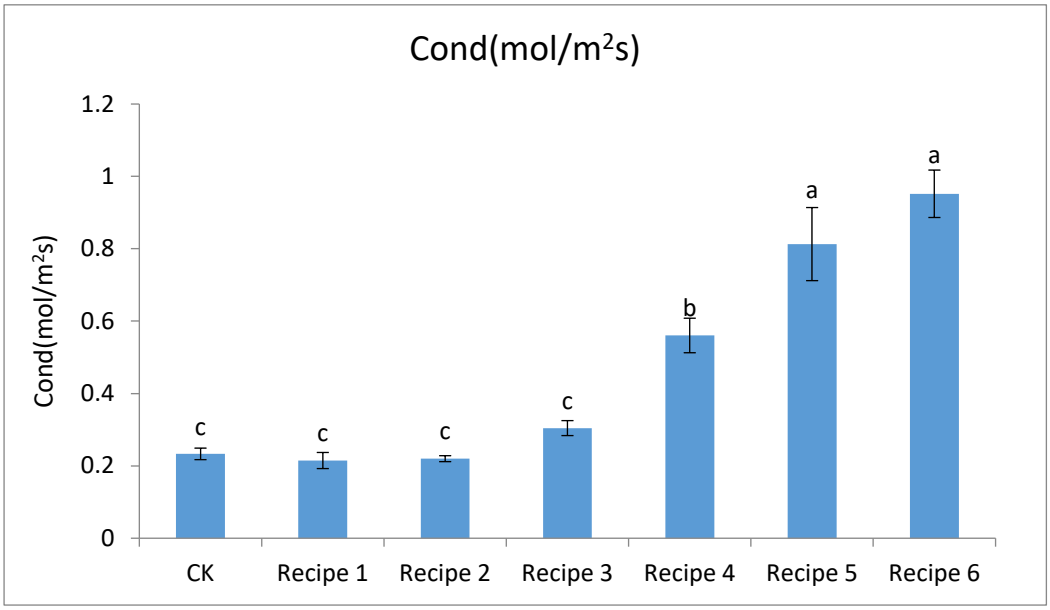


Figure 4. 2. 4 *The features of chlorophyll fluorescence reaction: Net photosynthetic rate (Pn); Actual photochemical efficiency of PS II (PhiPS2); Optimal photochemical efficiency of PS II (Fv/Fm); Photochemical quenching (qP); The labelled different letters are significantly different at P ≤0.05, n=3.*

In general, monochromic red light demonstrated a remarkably negative effect on PSII center activity, and monochromic blue light can heighten the PS II stress tolerance, as PS II conferred the best photochemical potential under 100% blue light. However, the electron production yield still possessed the highest efficiency from the combined spectrum, Recipe 3 had the highest photochemical efficiency, and Recipe 4 had the highest carbon assimilation. CK showed better performance than most LED recipes.

The gas exchange results for different LED recipes effects have detailed in Figure 4. 2. 5. CK demonstrated the lowest reading in both measurements, and the volume increased as the blue light proportion extending. This positive linear correlation indicated the improvement of blue light on stomatal movement.



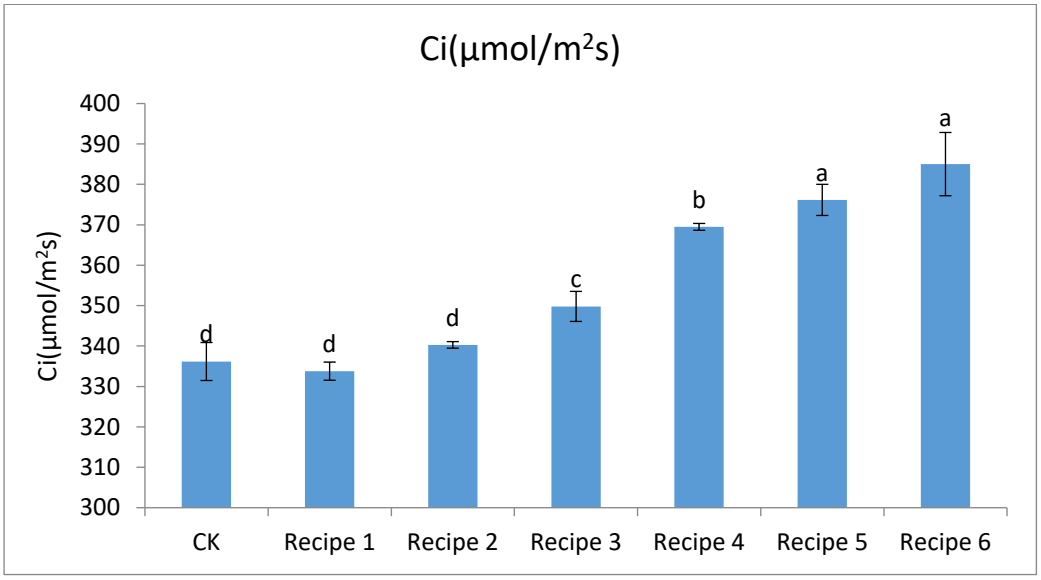


Figure 4. 2. 5 *Recipe1=100% red: 0%blue; Recipe 2=77% red: 23% blue; Recipe 3=73% red: 27% blue; Recipe 4=70% red: 30% blue; Recipe 5=62% red: 38% blue; Recipe 6=0% red: 100% blue; CK: Fluorescent lamp. Stomatal movement measurement for different LED recipes: Cond (stomatal conductance) and Ci (intercellular CO2 concentration). The labelled different letters are significantly different at P ≤0.05, n=3.*

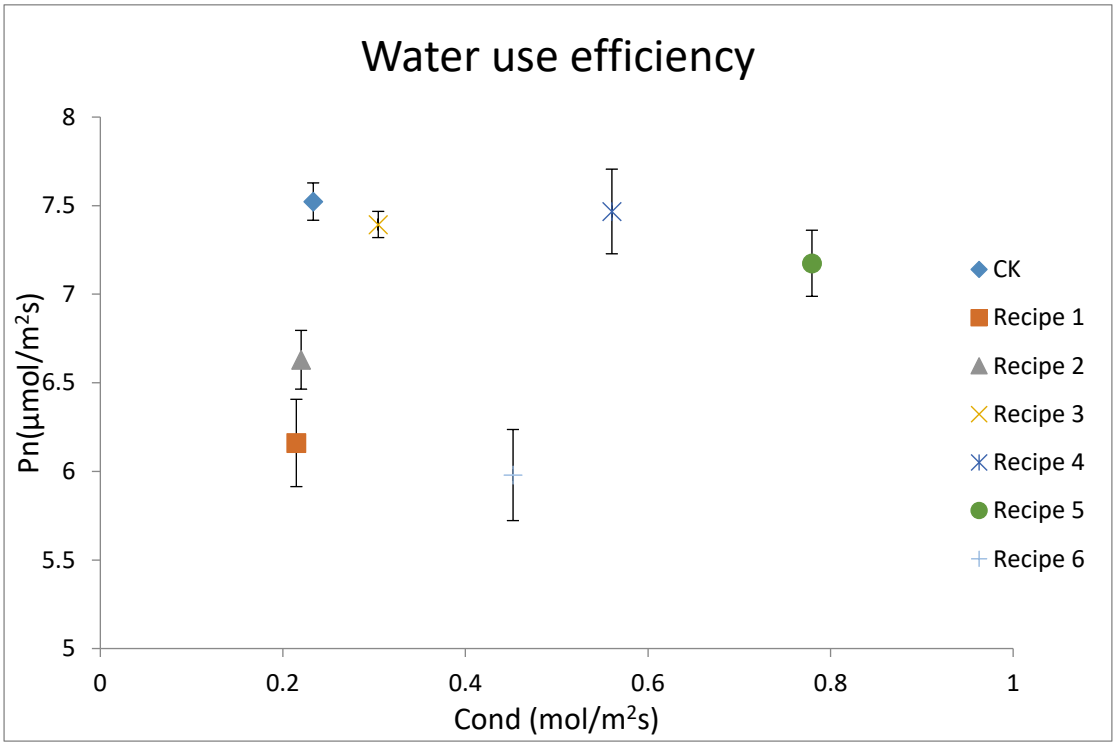


Figure 4. 2. 6 *The water use efficiency of different LED recipes treated pak choi. Calculated as net photosynthetic rate compared with stomatal conductance. The error bars are standard deviation values (n=3).*

Figure 4. 2. 6 displayed the water use efficiency (WUE) of different LED recipes treated pak choi, as it calculated from Pn (net photosynthetic rate) to Cond (stomatal conductance), the slope indicated the carbon fixed by each unit of water consumption. Although CK had average level of Pn, its extreme low Cond showed the highest WUE compared with all other LED recipes. Recipe 3 and Recipe 2 had similar WUE, and it is vital to notice that recipe 4 with the highest Pn revealed a similar WUE with Recipe 1, which had the lowest Pn in all LED recipes. As the stoma was forced to open, associating the increasing blue light proportion, even though Recipe 3 and 4 showed enhanced PhiPS2 and Pn, the WUE were still reduced.

In general, blue light played the most critical role in pak choi stomatal movement; with the opened stoma, the CO_2 absorption and transpiration were enhanced, which caused Ci to increase. On the one hand, the excessive intercellular carbon dioxide concentration indicated that the excessively absorbed carbon dioxide could not be consumed entirely by the Calvin cycle. On the other hand, the evaporation of water caused by transpiration is increase, resulting in lower water use efficiency.

4. 2. 3. Different photosynthetic response in four pak choi varieties

The four varieties of pak choi (802 Bushido, Kungfu 2, 702 Warrior and Green Hill 402) were grown under the following conditions for 30 days: red/blue: 70%/30% LED recipe at 130 μmol/m^2s. Growth status was measured periodically. Fluorpen FP100 was used on the 10^{th} day, 20^{th} day and 30^{th} day after transplanting for optimal photochemical efficiency, and Li-6400 XT was used on the 15^{th} day and 30^{th} day for both gas exchange and chlorophyll fluorescence measurement.

Figure 4. 2. 7 demonstrated the maximum photochemical efficiency of PSII during growth. For measurement on the 10^{th} day and 20^{th} day there was no significant difference observed in all treatments, but on the 30^{th} day the 802 Bushido (Led 1) and Kungfu 2 (Led 2) showed reduced readings, which indicated the stressed PS II center. Simultaneously, 802 Bushido and Kungfu 2 with fluorescent lamp treatment (CK 1 and CK 2) still maintained high readings compared with other treatment. The rest of the LED treatment and CK had no significant difference through all measurements.

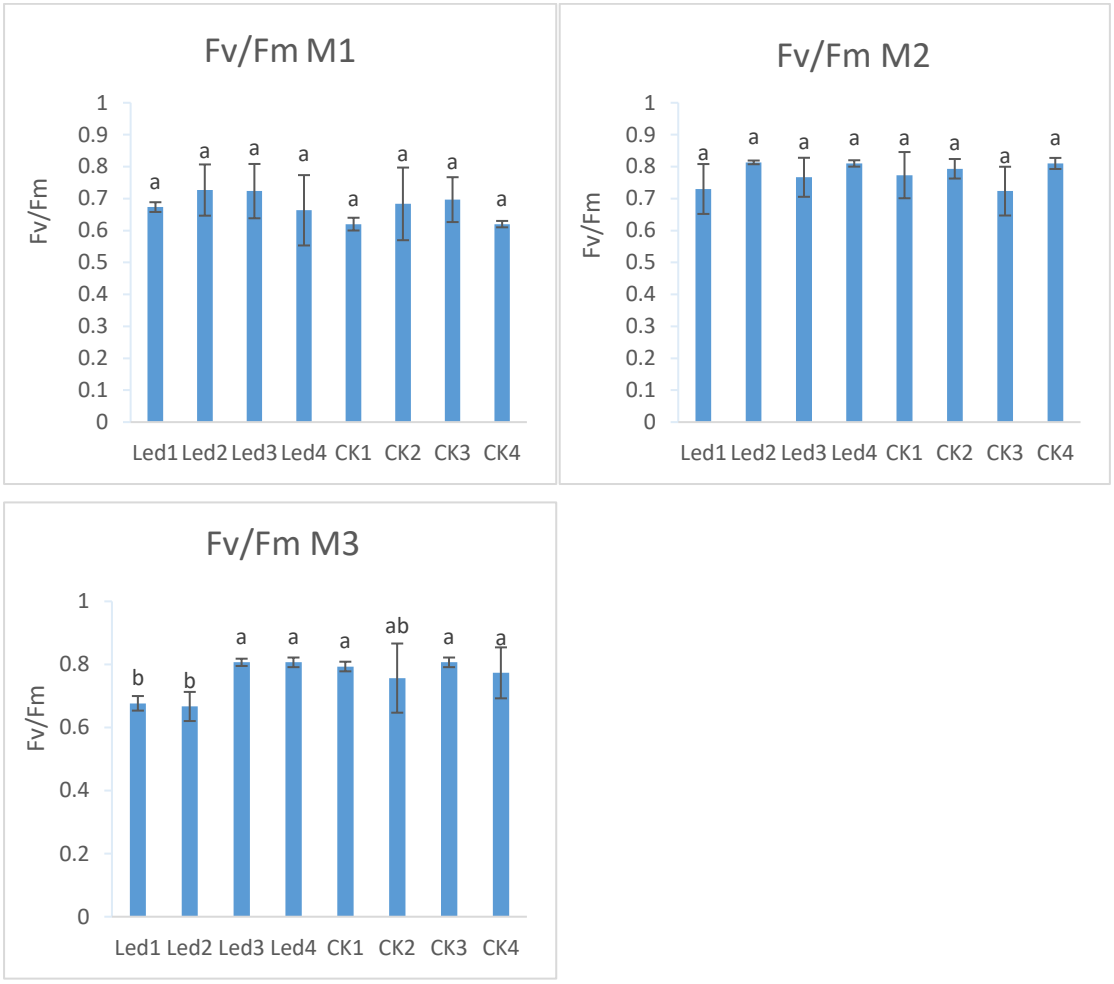
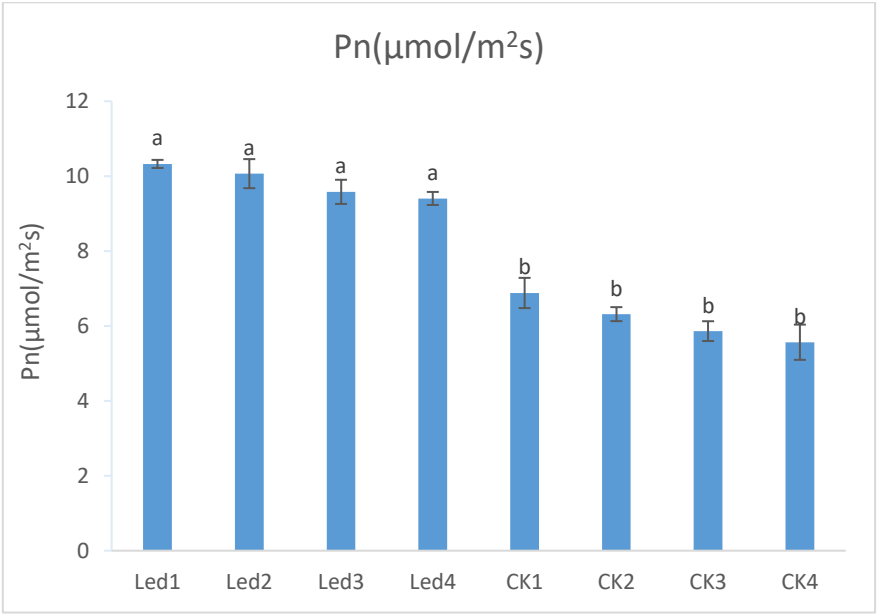
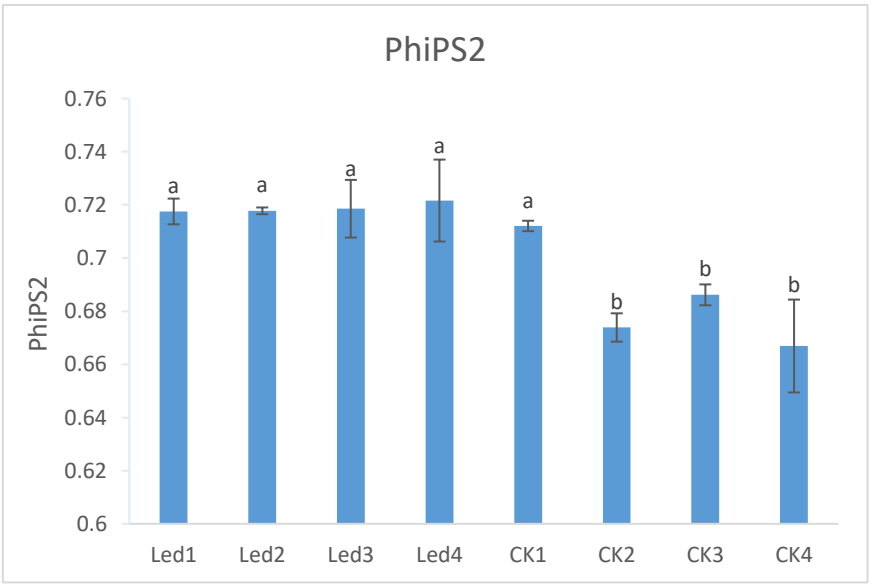


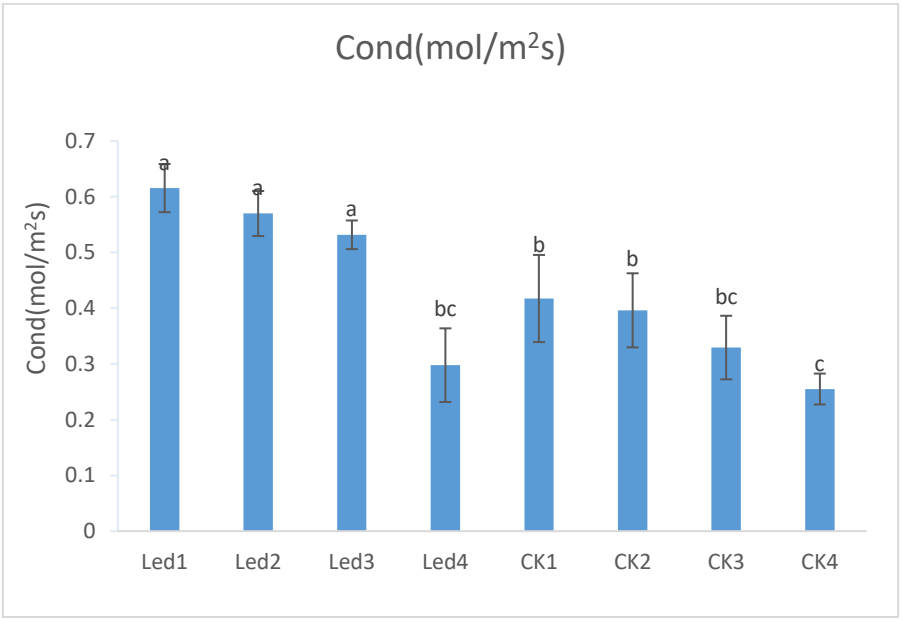
Figure 4. 2. 7 *The maximum photochemical efficiency of PSII (Fv/Fm) of four pak choi varieties (Led1-Led 4 stand for 802 Bushido, Kungfu 2, 702 Warrior and Green Hill 402 treated by red/blue: 70%/30% LED recipe; while CK1-4 stand for the four varieties treated by fluorescent lamp as CK), measured at M1 refers to 10th, M2 refers to 20th and M3 refers to 30th day, respectively. The labelled different letters are significantly different at P ≤0.05, n=3.*

The observation mentioned above indicated that, as maximum photochemical efficiency of PSII will not be affected by growing conditions in most cases, so it can be summarized to represent the degree of stress on PSII. The results determined that the status of PSII was changing during the entire growth process, and the tolerance between each variety was different, which proved

as 802 Bushido and Kungfu 2 was reducing at the ending of the growth period. Generally, this parameter should locate the range around 7.5-8.0, and these four varieties maintain the adaptability to LED lighting sources.







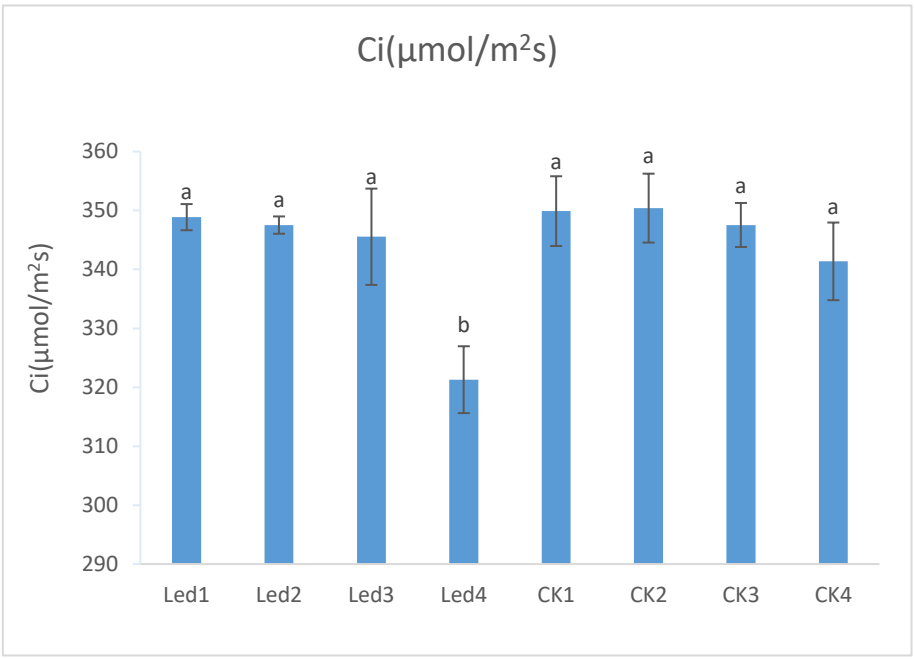
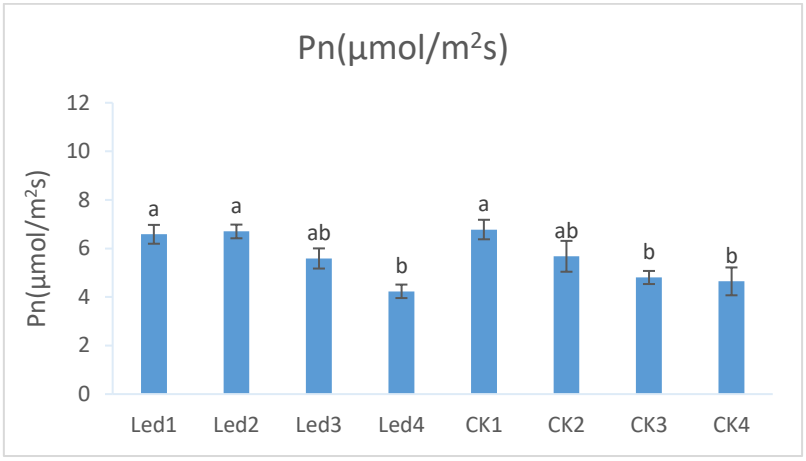


Figure 4. 2. 8 *The gas exchange and chlorophyll fluorescence measurements for 4 varieties at 15th day. Led1-Led 4 stand for 802 Bushido, Kungfu 2, 702 Warrior and Green Hill 402 treated by red/blue: 70%/30% LED recipe for 15 days growth, while CK1-4 stand for the four varieties treated by fluorescent lamp as CK. The labelled different letters are significantly different at P ≤0.05, n=3.*

Figure 4. 2. 8 demonstrated the photosynthetic characteristics of four pak choi varieties. There were no significant ($P>0.05$) differences between each variety, either treated by LED recipes or fluorescent lamp, but all LED recipes showed higher Pn than CK. Meanwhile, PhiPS2 had no difference among varieties when treated by the LED recipe as well ($P>0.05$), but in CK 802, Bushido had significantly higher results compared with other CK ($P < 0.05$). In stomatal conductance measurements. Green Hill 402 showed notably reduced responses in stomatal conductance measurements compared with other varieties, and all LED treatments showed higher results than CK ($P < 0.05$). However, there was no significant difference between all treatments in Ci, except Green Hill 402 reached the lowest when treated by LED recipes.

In general, there were no significant differences ($P>0.05$) among these four varieties in Pn and PhiPS2, except 802 Bushido remained high performance both in LED treatment and CK, which had significantly higher volume in PhiPS2 compared with other varieties when treated by the fluorescent lamp ($P < 0.05$). Nevertheless, Green Hill 402 showed negative reply in a stomatal movement when treated both by LED and CK, which had the lowest Cond among all treatments. The results indicated that even theses four varieties had changed Cond refers to different CO_2 absorption rate, they still represented a similar carbon dioxide assimilation rate (Pn). As all LED treatments showed better PhiPS2 as CK, it suggested that the efficiency of photosystem II was the critical factor for yield production among the changing light conditions.


Pn(μmol/m²s)
Pn(μmol/m²s)
12
10
8
6
4
2
0
a
a
ab
b
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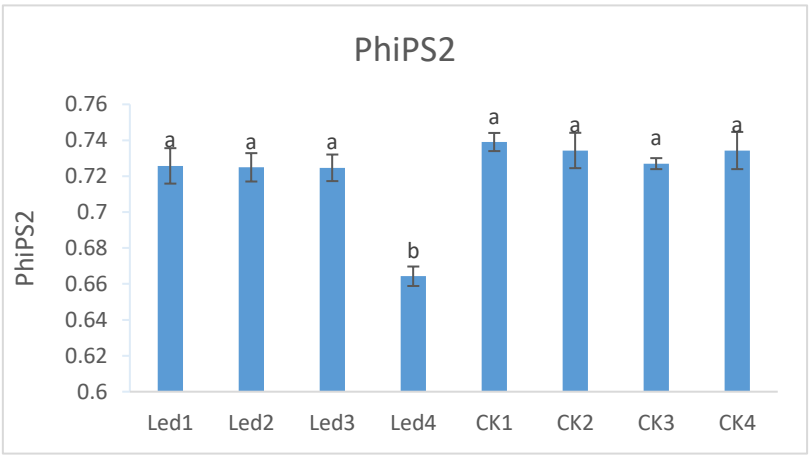
Led2
Led3
Led4
CK1
CK2
CK3
CK4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0.7
0.68
0.66
0.64
0.62
0.6
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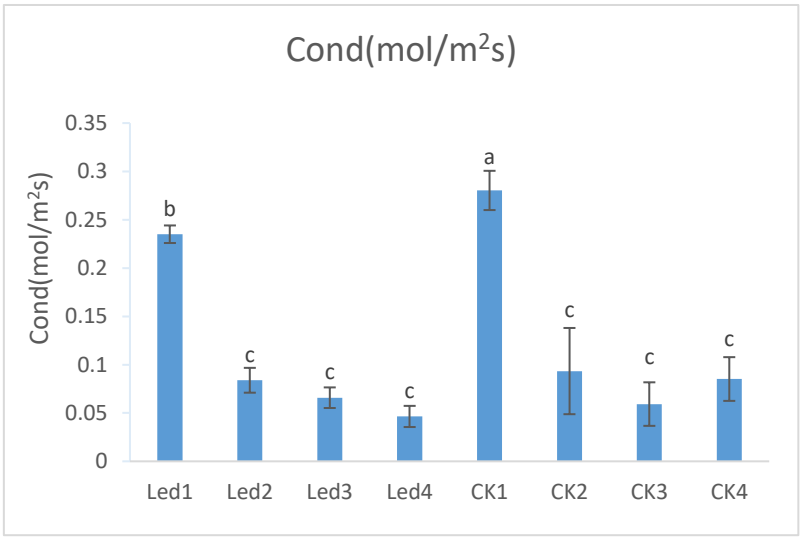
Led3
Led4
CK1
CK2
CK3
CK4


Cond(mol/m²s)
Cond(mol/m²s)
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0.3
0.25
0.2
0.15
0.1
0.05
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CK1
CK2
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CK4

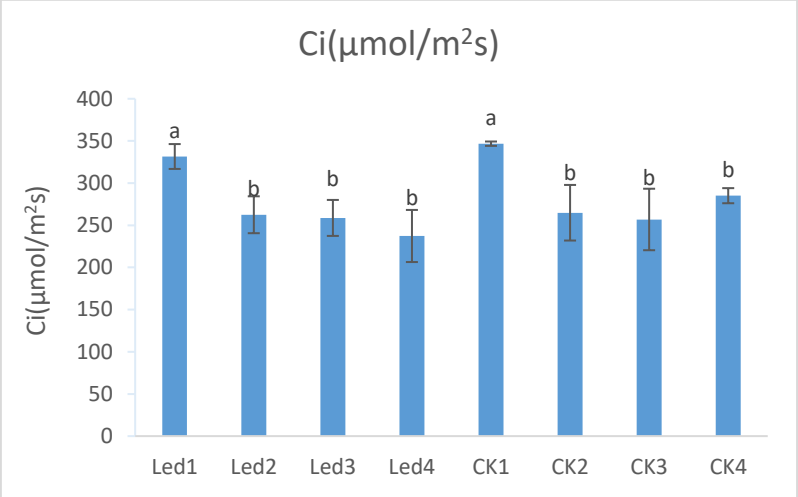


Figure 4. 2. 9 *The gas exchange measurements results. Led1-Led 4 stand for 802 Bushido, Kungfu 2, 702 Warrior and Green Hill 402 treated by red/blue: 70%/30% LED recipe for 30 days growth, while CK1-4 stand for the four varieties treated by fluorescent lamp as CK. The labelled different letters are significantly different at P ≤0.05, n=3.*

After 30 days of growth, the gas exchange and chlorophyll fluorescence were measured before harvested (Figure 4. 2. 9). 802 Bushido and Kungfu 2 had the highest Pn than all other varieties, followed by 702 Warrior and Green Hill 402. There were no significant differences between LED treatment and CK, and the plants with CK had a related tendency. Nevertheless, the PhiPS2 present almost the corresponding level among the varieties except for Green Hill 402 ($P>0.05$), which had a 10% lower reading compared with others ($P<0.05$). The rest varieties presented no differences ($P>0.05$). For Cond measurement, 802 Bushido had dramatically higher level both in LED treatment and CK ($P<0.05$), the others had no significant differences ($P>0.05$). Ci showed comparable results, as 802 Bushido reached the peak in both conditions.

In conclusion, the carbon dioxide assimilation was performed differently

between the varieties when the measurement was conducted on the 30th day, as 802 Bushido had the highest Pn, followed by Kungfu 2, 702 Warrior and Green Hill 402. Contrary to the previous results, Kungfu 2 had reduced PhiPS2 reading in LED treatment but enhanced CK, intimating that Green Hill 402 can have more prolonged activity in photosystem II with fluorescent lamp. For stomatal conductance, 802 Bushido had significantly higher performance than any others in both LED and CK treatment, as the increasing in Pn was not so significantly, this performance was led to lower water use efficiency.

4. 3 Transcriptomic analysis of Pak Choi grown under different LED light spectra

Based on previous studies, RNA Sequencing technology was used to identify gene expression profiling and significant differential expressed genes and gene regulatory networks associated with pak choi plant growth and development under different LED light spectra.

4. 3. 1 RNA sequencing results

We obtained an average of 47 million trimmed reads from each sequencing samples with the transcriptome data analysis. Approximately 37 million reads from each sample were mapped to the reference genome, accounting for 85% of the total reads. Above that, approximately 33 million reads were uniquely and correctly mapped *Brassica rapa* genome, which showed in Table 4.3.1.

To assess transcriptome similarity between samples, principal component analysis (PCA) and hierarchical clustering was performed (Figure 4 .3. 1 and Fig 4. 3. 2). Both analyses revealed discrete clustering of samples according to different treatments and biological replicates, meaning some high similarity between the replicate samples under LED light spectra but some variations within replicates. Therefore, the reads were further trimmed by CLC, and results have been showing in Figure 4. 3. 2 and Figure 4. 3. 3.

Table 4. 3. 1 *Read alignment of RNA-seq data between different lighting recipes, refers to Brassica rapa FPsc_v1.3 genome. B refers to 100% blue light treatments, M refers to red/blue: 70%/30% treatments, R refers to 100% red light treatments, CK refers to fluorescent treatments. 1, 2 and N refer to different replicates, respectively.*

Sample	Trimmed Reads	Mapped Reads		Uniquely and Correctly Mapped Reads	
		Count	Percentage	Count	Percentage
B1	51,857,862	44,305,423	85.44%	37,272,202	71.87%
B2	42,654,370	36,328,230	85.17%	30,561,758	71.65%
BN	48,932,560	41,861,176	85.55%	34,962,904	71.45%
CK1	44,261,302	38,026,211	85.91%	31,988,596	72.27%
CK2	46,758,818	40,070,136	85.70%	33,564,240	71.78%
CKN	48,722,188	41,750,908	85.69%	35,317,506	72.49%
M1	52,359,214	44,755,214	85.48%	37,701,050	72.00%
M2	52,908,140	45,223,781	85.48%	37,915,182	71.66%
MN	47,293,482	40,282,678	85.18%	33,872,902	71.62%
R1	44,082,194	37,612,570	85.32%	31,431,574	71.30%
R2	41,435,294	35,446,888	85.55%	29,839,970	72.02%
RN	44,950,492	38,477,686	85.60%	32,283,156	71.82%

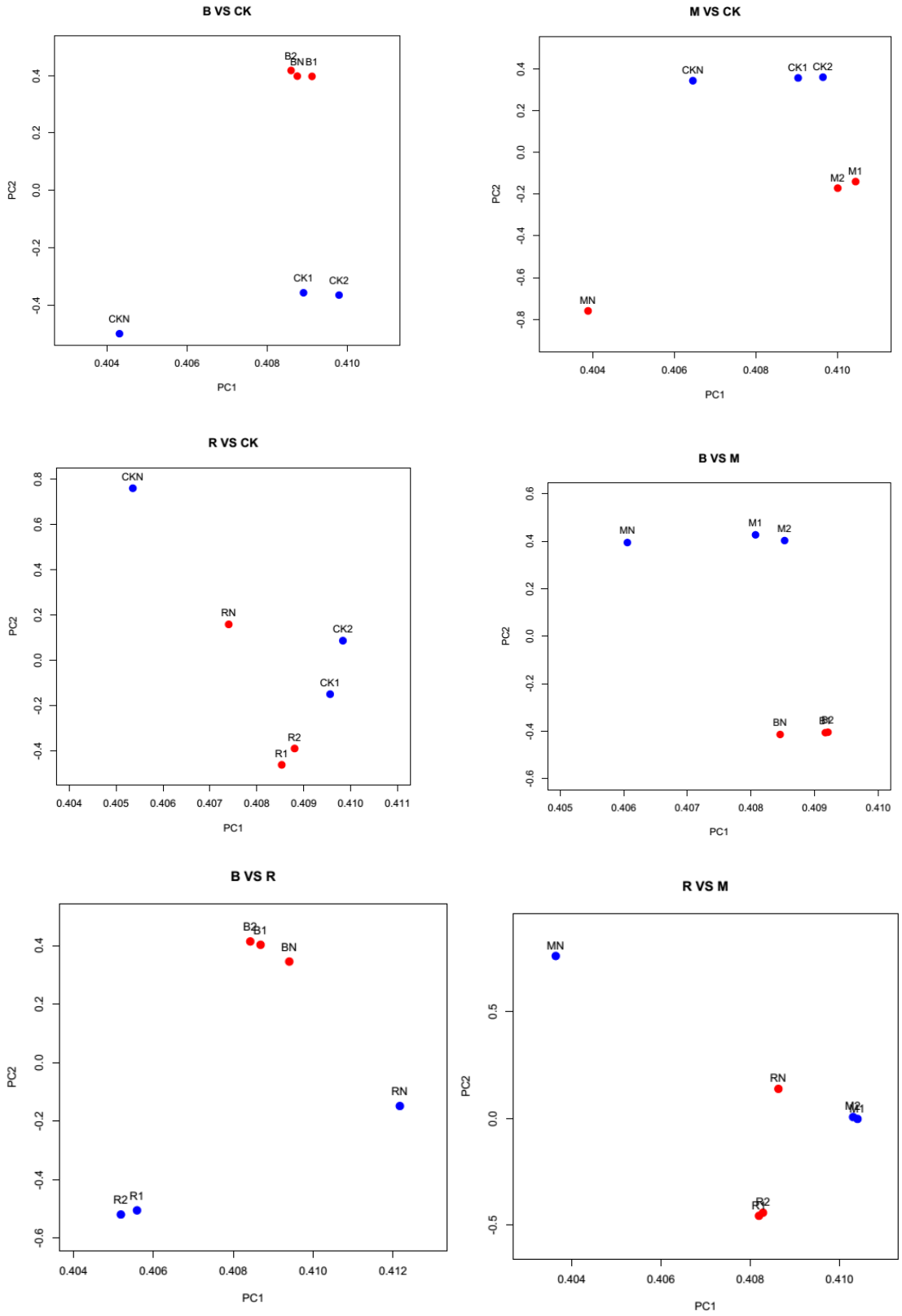


Figure 4. 3. 1 *The Principal Component Analysis (PCA) for RNA seq results*

The number of reads was significantly decreasing after trimming by CLC, especially the saturation evaluation of libraries was reduced significantly, which led to the shortage of gene expression coverage. On the other hand, the quality distribution of trimmed reads was dramatically improved, which demonstrated more improved PCA results in Figure 4. 3. 2. Beforehand, it displayed the differential expressed gene level, as blue light treatments had the most notable differences compared with other treatments.

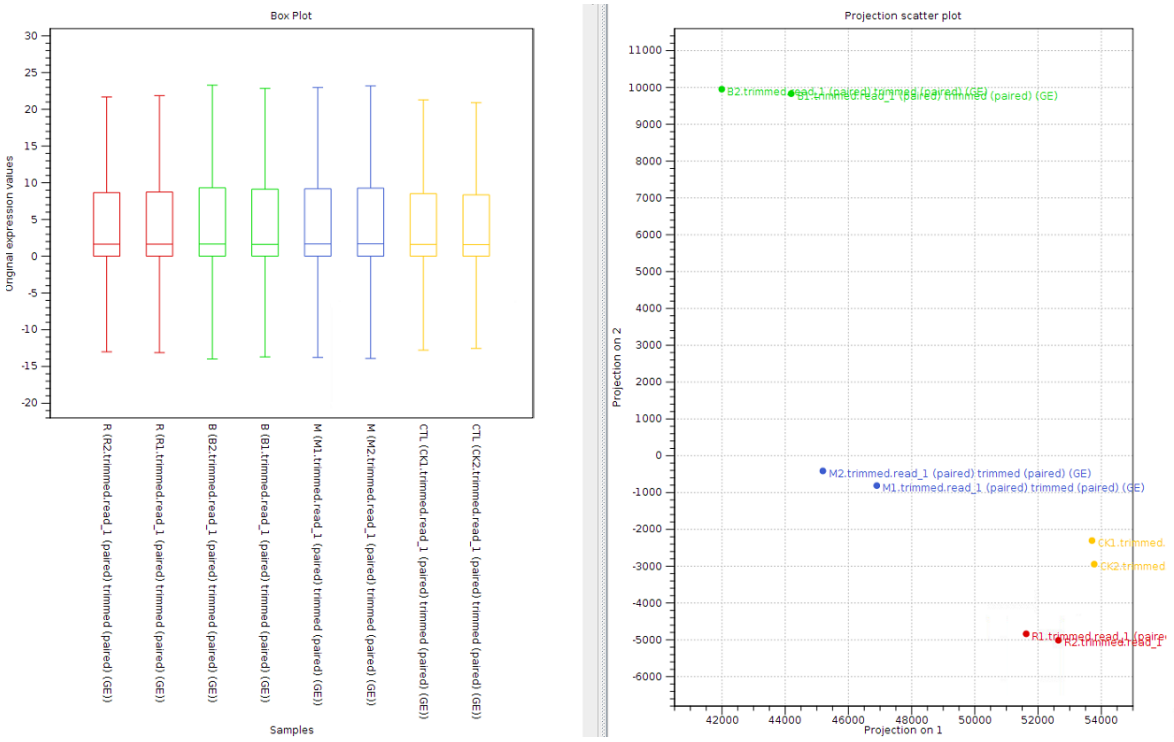
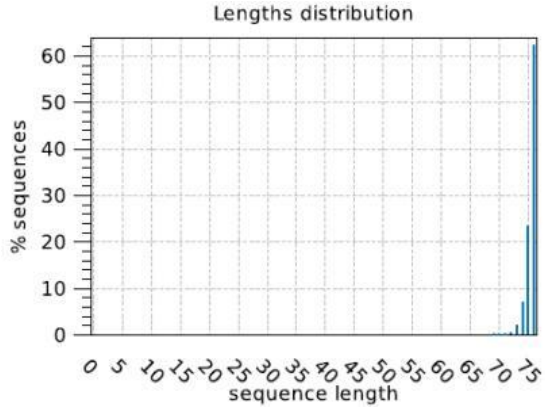
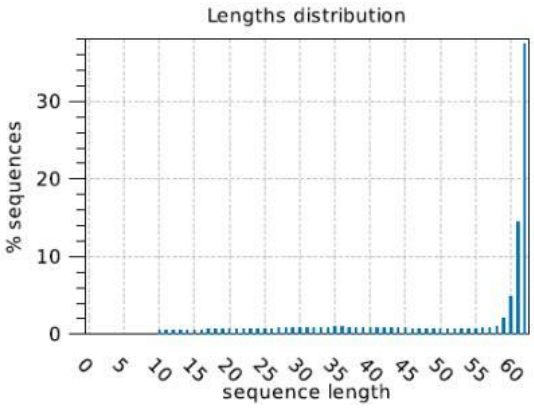
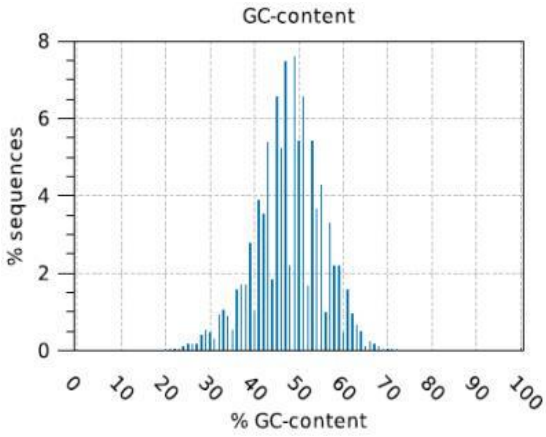
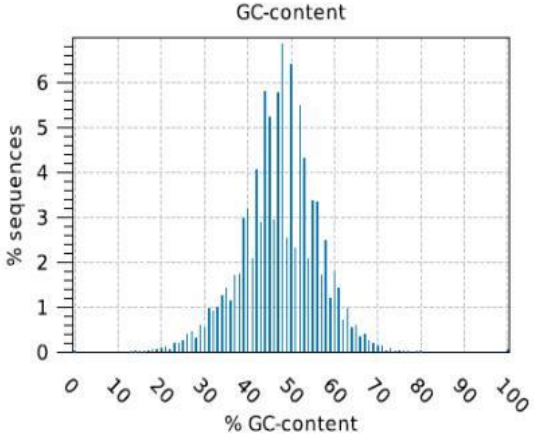


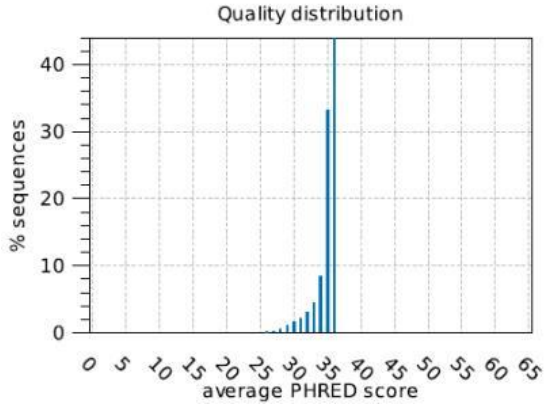
Figure 4. 3. 2 *The PCA results and Box Plot after further trimming by CLC*

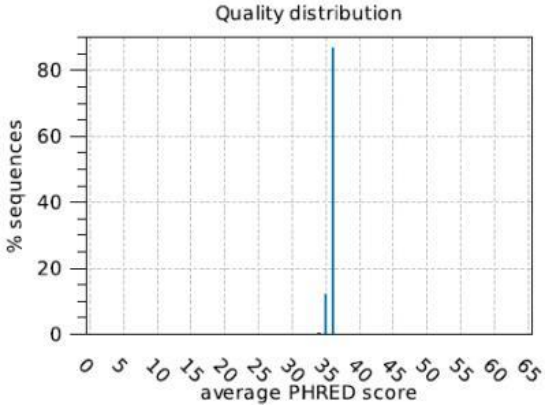

Lengths distribution
% sequences
sequence length


Lengths distribution
% sequences
sequence length


GC-content
% sequences
% GC-content


GC-content
% sequences
% GC-content


Quality distribution
% sequences
average PHRED score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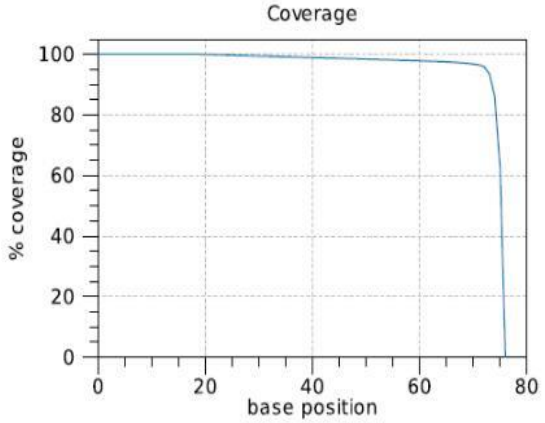
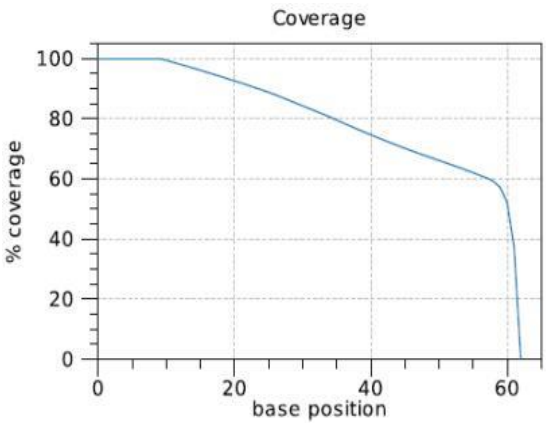

Coverage
% coverage
base position


Coverage
% coverage
base position

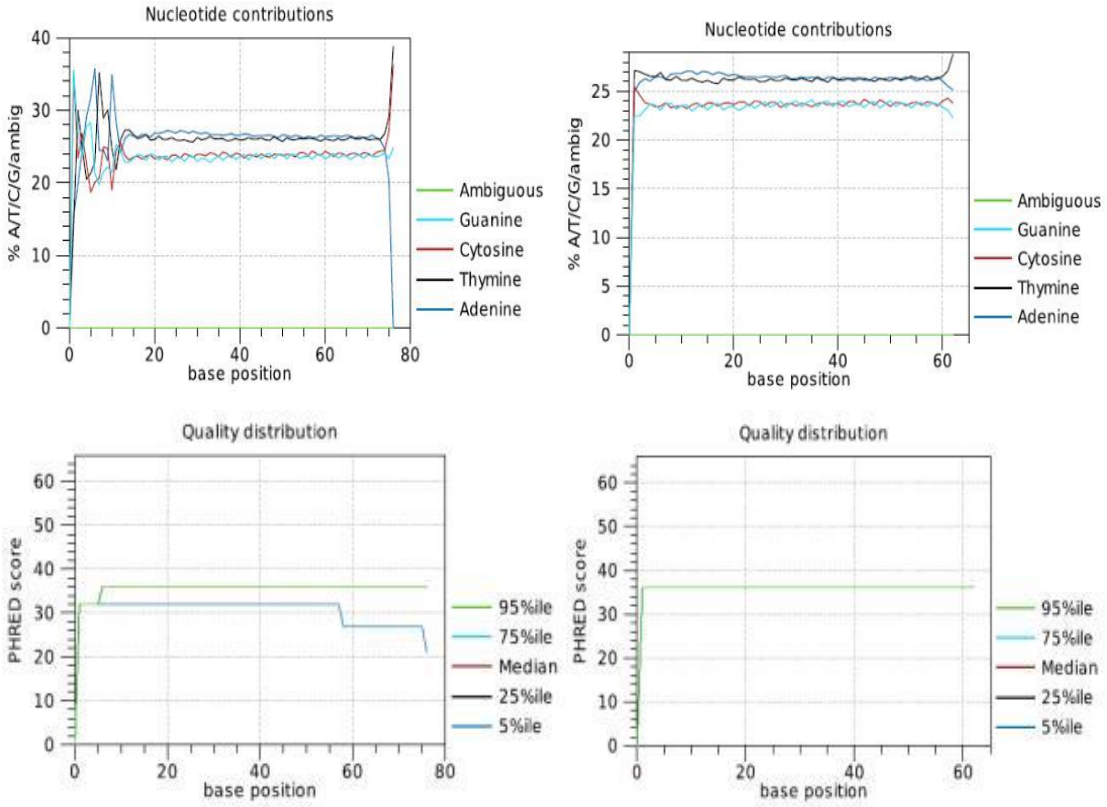


Figure 4. 3. 3 *The reads distribution of RNA sequencing results, in same line from left to right refers to before/after CLC trimming.*

After the normalization, according to the sequencing principle, the reads will increase significantly when the expression level of a particular gene is high. However, if the length of a gene is long, the number of reads will correspondingly increase. In order to reasonably compare gene expression levels, reads are calculated by using RPKM:

$$RPKM = 10^9 C/NL$$

While C is the number of uniquely read reads aligned to the reference gene, N is the total number of reads that are uniquely aligned to all genes, and L is the number of gene bases. Thus, it can directly examine the differences in gene expression between samples.

After the calculation, there were a total of 41020 genes identified. Above that, FDR<0.05 and $|\log_2 Ratio| \geq 1$ were considered as differential expressed gene (DEG), compared with CK. As results, there were 911 DEGs in Recipe M, with 447 down-regulated and 464 up-regulated; 2451 DEGs in Recipe B, with 1410 down-regulated and 1041 up-regulated; and 269 DEGs in Recipe R, with 183 down-regulated and 86 up-regulated were identified. (Figure 4. 3. 4), which demonstrated that the pak choi with blue light treatment showed a remarkable higher expression quantity than any other treatments.

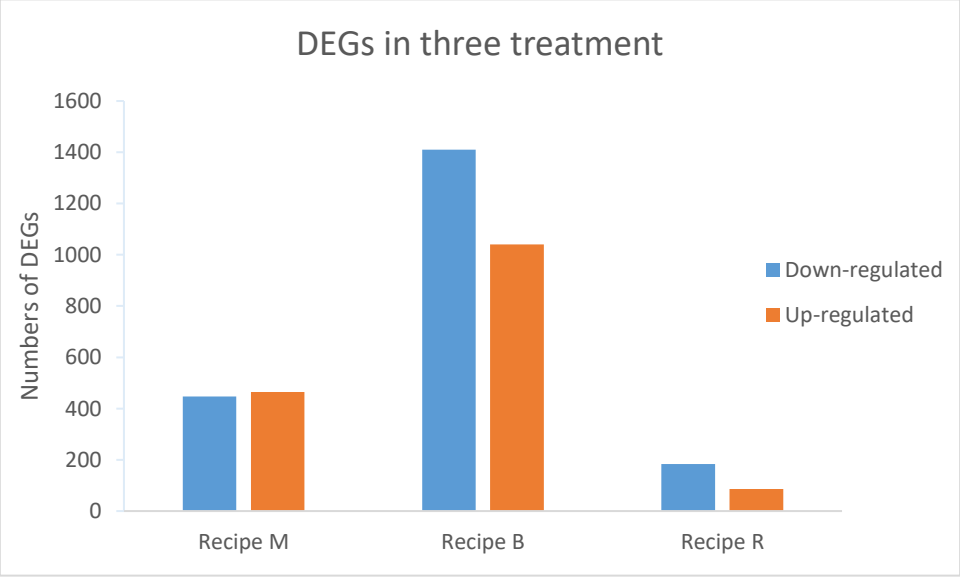


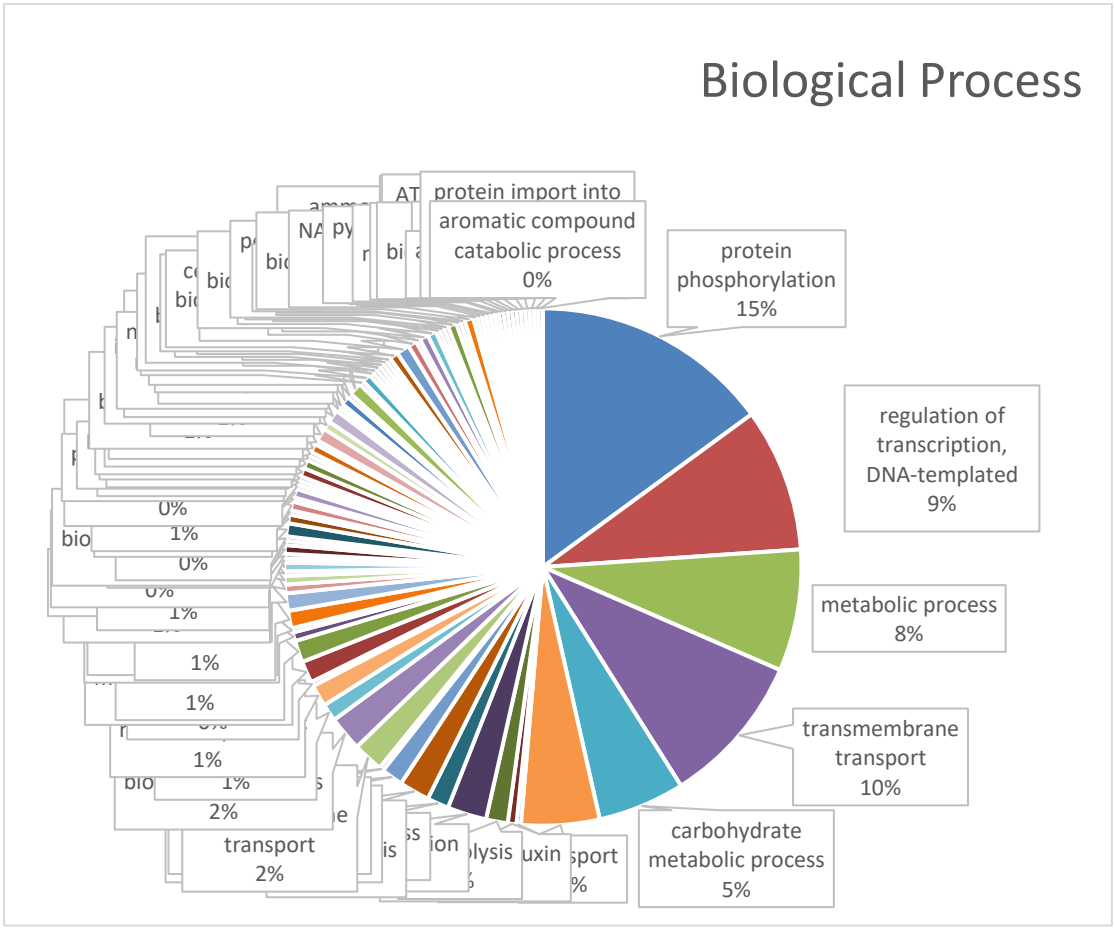
Figure 4. 3. 4 *The differential expressed genes in three treatment compared with CK (Recipe M: 70% red / 30% blue; Recipe B: 100% blue; Recipe R: 100% red).*

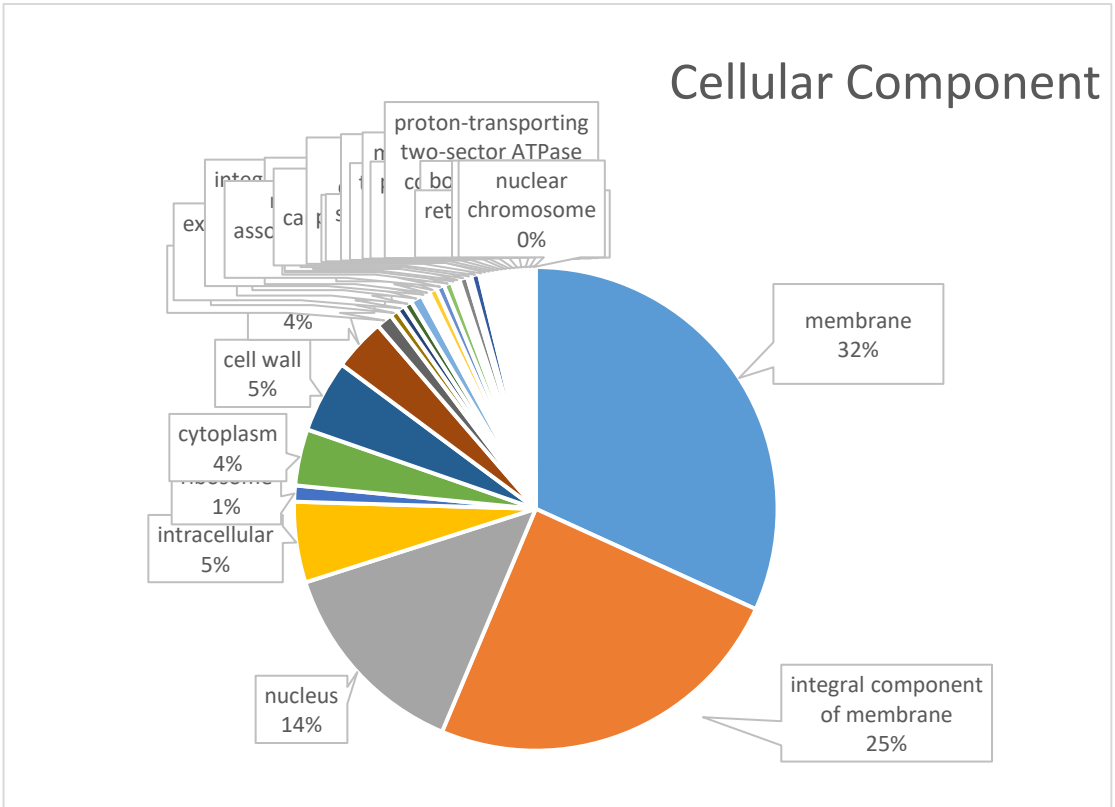
To further understand the gene function classification, the Gene Ontology (GO) analysis was applied. GO results were displayed in three categories (Figure 4. 3. 5):

Biological Process: 15% located in protein phosphorylation, 9% in the regulation of transcription, 8% in metabolic process, 10% in transmembrane transport, and 5% in carbohydrate metabolic process.

Cellular Component: 15% in the membrane, 25% in integral component of membrane, 14% in the nucleus, 5% in the intercellular, 4% in the cytoplasm, and 5% in the cell wall.

Molecular Function: 9% in protein binding, 7% in ATP binding, 6% in protein kinase activity, 5% in DNA binding, 4% in oxidoreductase activity, 4% in heme binding, 3% in transcription factor activity, sequence-specific DNA binding, 3% in iron ion binding, and 3% in oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen.


Biological Process
protein import into aromatic compound catabolic process 0%
protein phosphorylation 15%
regulation of transcription, DNA-templated 9%
metabolic process 8%
transmembrane transport 10%
carbohydrate metabolic process 5%
transport 2%
0%
1%
0%
1%
1%
1%
2%
1%
1%
1%
2%


Cellular Component
proton-transporting two-sector ATPase
nuclear chromosome 0%
membrane 32%
integral component of membrane 25%
nucleus 14%
intracellular 5%
1%
cytoplasm 4%
cell wall 5%
4%

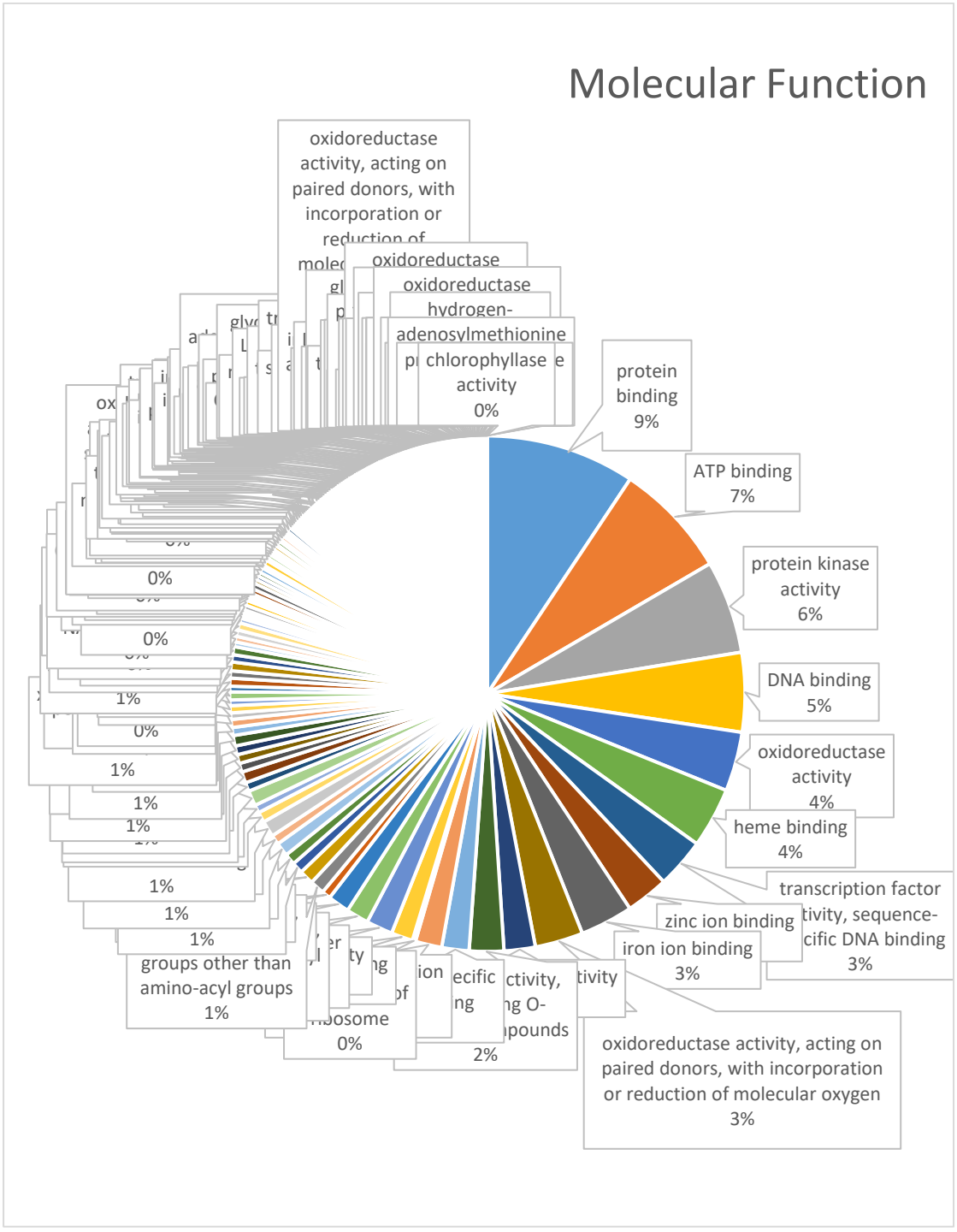


Figure 4. 3. 5 *Gene Ontology (GO) function enrichment analysis of LED light treated pak choi gene expression.*

In order to further understand the biological functions of plant growth and photosynthesis-related genes under three LED light treatments, the differential expressed genes were further analyzed by metabolic pathway enrichment using the KEGG database. Pathway Significant Enrichment Analysis applies a hypergeometric test to find KEGG Pathway significantly enriched in differential expression compared to the entire genomic background (Figure 4. 3. 6).

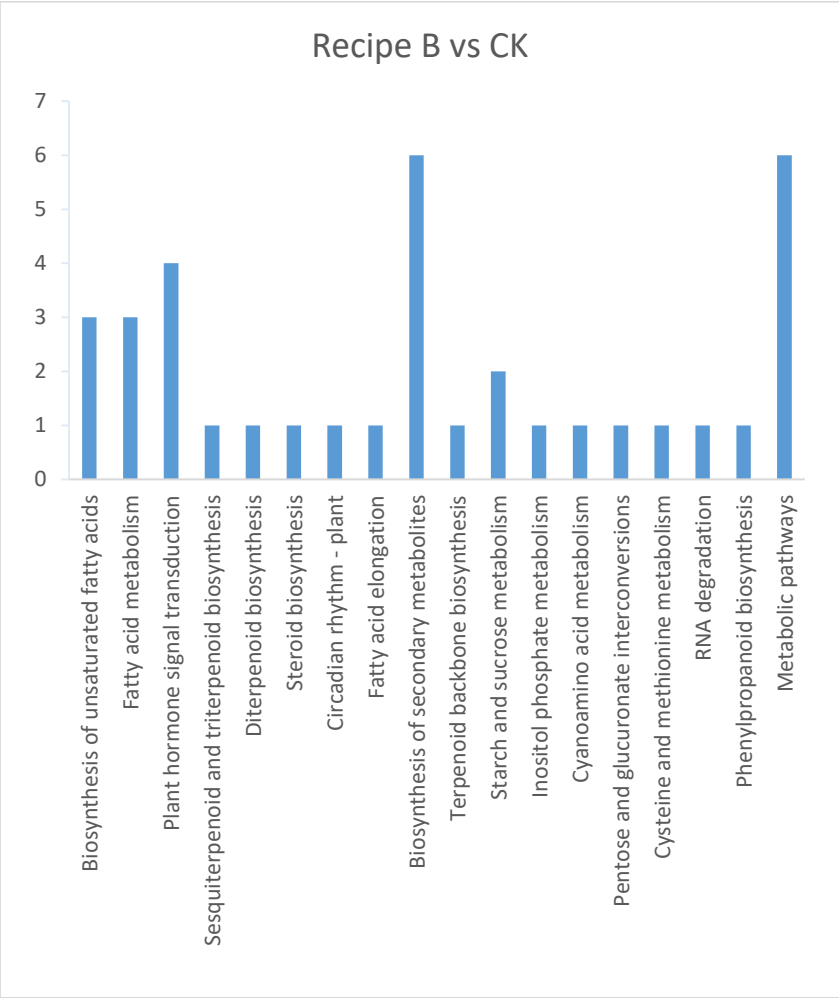


Figure 4. 3. 6 *KEGG pathway function enrichment analysis of differential expressed genes between each LED recipe treatment and CK.*

It demonstrated that in all three LED treatment, both the biosynthesis of secondary metabolites and metabolites pathways is higher than the others, including the plant's hormone signal transduction pathway. It is worth notice that biosynthesis of unsaturated fatty acids and Fatty acid metabolism pathway were more activated in Recipe B treatment, which may indicate that the wax biosynthesis for leaf surface coverage was improved by blue light, and it may lead to higher cold resistance and pest defence (Ishizaki N. et al., 1996).

As the very first and essential photoreceptor, the genes that related to photosystem II and photosystem I, especially the antenna protein, were observed in Table 4.3.2

Table 4. 3. 2 *The selected differential expressed genes which related to photosystem and light harvesting complex in LED light treated pak choi, compared with CK in* log_{10} *fold change.*

Feature ID	R vs CK Fold change	p- value	B vs CK Fold change	p- value	M vs CK Fold change	p- value
Brara.B01738	-1.39	0.00	-1.75	0.00	-1.64	0.00
Brara.A00858	-1.28	0.96	-1.32	0.15	-1.56	0.00
Brara.A01166	-1.24	0.00	-1.56	0.00	-1.51	0.00
Brara.J00193	-1.11	0.91	-1.70	0.00	-1.50	0.00
Brara.H00652	-1.15	0.05	-1.57	0.00	-1.30	0.00
Brara.F03187	-1.15	0.01	-2.57	0.00	-1.45	0.00
Brara.F00021	-1.17	0.00	-2.14	0.00	-1.41	0.00
Brara.H02525	-1.08	1.00	-4.20	0.00	-1.41	0.00
Brara.I03747	-1.18	0.01	-1.64	0.00	-1.40	0.00

Brara.G00547	-1.20	0.00	-2.07	0.00	-1.40	0.00
Brara.H02384	-1.08	1.00	1.08	1.00	-1.36	0.00
Brara.C04035	-1.06	0.70	-1.81	0.54	-1.36	0.00
Brara.B03307	-1.08	1.00	-1.00	1.00	-1.32	0.00
Brara.I00765	-1.19	0.00	-1.39	0.00	-1.32	0.00
Brara.E02107	-1.19	0.29	-1.76	0.00	-1.32	0.00
Brara.I02857	1.09	1.00	-2.23	0.00	-1.29	0.00
Brara.I02817	-1.18	0.00	-1.66	0.00	-1.29	0.00
Brara.C03812	-1.17	0.01	-1.74	0.00	-1.29	0.00
Brara.A00845	-1.07	1.00	-1.71	0.00	-1.27	0.00
Brara.I04910	-1.12	0.35	-2.25	0.00	-1.27	0.00
Brara.J00467	-1.25	0.01	-1.03	1.00	-1.25	0.00
Brara.G00467	-1.06	1.00	-1.72	1.00	-1.25	0.00
Brara.E03123	-1.11	1.00	-1.43	0.00	-1.24	0.00
Brara.F01095	-1.03	1.00	-2.32	0.00	-1.23	0.00
Brara.D02090	-1.00	1.00	-1.40	0.00	-1.22	0.01
Brara.A02793	-1.14	0.57	-1.56	0.00	-1.22	0.00
Brara.H01891	-1.16	0.01	-1.54	0.00	-1.22	0.00
Brara.F00256	-1.09	1.00	-3.30	0.00	-1.21	0.02
Brara.I04677	1.01	1.00	1.00	1.00	-1.21	0.01
Brara.F02286	-1.04	1.00	-1.41	0.00	-1.21	0.00
Brara.E00101	-1.51	0.00	1.15	0.02	-1.20	0.00
Brara.H02952	-1.07	0.99	-1.25	0.00	-1.20	0.00
Brara.E01452	-1.05	1.00	-1.22	0.00	-1.20	0.00
Brara.I00297	-1.05	1.00	-1.25	0.00	-1.20	0.00
Brara.H00553	-1.10	0.00	-1.37	0.00	-1.18	0.00
Brara.E01277	-1.16	0.00	-1.14	0.00	-1.17	0.00
Brara.J00907	1.05	1.00	-2.20	0.00	-1.17	0.00
Brara.D02820	-1.15	0.00	-1.40	0.00	-1.17	0.00
Brara.F00523	-1.12	0.01	-1.44	0.00	-1.16	0.00
Brara.D01828	-1.12	0.34	-1.32	0.00	-1.16	0.01
Brara.B00018	-1.00	1.00	-2.24	0.00	-1.16	0.02
Brara.G01647	-1.05	1.00	-1.51	0.00	-1.16	0.00
Brara.H00039	-1.06	1.00	-1.27	0.00	-1.15	0.00
Brara.H00722	-1.08	1.00	-1.64	0.00	-1.15	0.02

Brara.C02638	-1.11	0.14	-1.17	0.00	-1.15	0.00
Brara.I02744	-1.03	1.00	-1.24	0.00	-1.14	0.00
Brara.H01465	-1.07	1.00	-1.51	0.00	-1.14	0.04
Brara.I05310	-1.05	1.00	-1.62	0.00	-1.13	0.00
Brara.A02541	-1.09	0.98	-1.41	0.00	-1.13	0.02
Brara.I02349	-1.06	1.00	-1.12	0.00	-1.13	0.00
Brara.D00751	-1.12	0.00	-1.32	0.00	-1.13	0.00
Brara.E00573	-1.20	0.00	1.11	0.01	-1.12	0.01
Brara.F00205	-1.07	0.32	-1.38	0.00	-1.11	0.00
Brara.B03997	-1.02	1.00	-1.12	0.00	-1.10	0.01
Brara.H00177	1.10	1.00	-2.00	0.01	3.32	0.00
Brara.E01976	-1.18	1.00	23.82	0.00	3.89	0.00

As results, 56 significantly differentially expressed genes in Recipe M vs CK detected, 17 and 49 significantly differentially expressed genes in Recipe R vs CK and Recipe B vs CK detected, respectively. There were 54 genes down-regulated by Recipe M, 17 genes down-regulated by Recipe R, and 47 genes down-regulated by Recipe B. The annotations of genes in Table 5.3.2 have been pointing in appendix 2, and there were 16 of them related to photosystem II, 21 of them related to photosystem I, and 16 genes related to light-harvesting complex. It is conspicuous that Recipe M significantly up-regulated Brara.E01976, which coded ELIP (early light-induced proteins), and highly up-regulated by Recipe B. Although they were all downregulated by Recipe M, the rest of genes, as the $\log_{10}$ fold changes was less than -1.3, indicated that the differences are relative small.

To determinate effects from different LED spectrum on pak choi growth, especially in morphologies and stress tolerance, DEGs with p-value<0.05 and $|\log_{10} Ratio| \geq 2$ from each LED treatment compared with CK were collected,

which refers to a total number of 912 DEGs. Table 4. 3. 3 lists several highly performed and strongly regulated.

Table 4. 3. 3 *The comparison of highly performed or regulated genes between each LED treatment with CK*

Feature ID	R vs CK Fold change	p-value	B vs CK Fold change	p-value	M vs CK Fold change	p-value
Brara.H01691	2.68	1.00	13.88	0.00	12.81	0.00
Brara.C01573	1.54	1.00	-1.69	1.00	36.58	0.01
Brara.H00687	-1.71	1.00	8.54	0.00	7.91	0.00
Brara.C04537	1.59	1.00	5.60	0.00	3.83	0.00
Brara.F01137	1.19	1.00	-1.17	1.00	2.40	0.00
Brara.H01125	2.29	0.00	1.59	0.19	2.62	0.00
Brara.G03530	-1.45	0.07	2.03	0.00	-2.47	0.00
Brara.I00981	-2.52	0.00	-1.20	0.80	-4.34	0.00

Brara.H01691 was significantly up-regulated by Recipe M and B, compared with CK. It is annotated as H^+-ATPase 1 coding gene, located on plasma membrane for creating the electrochemical gradients; Brara.C01573 was highly performed in Recipe M but no significant difference in Recipe R and B. It is annotated as cytochrome P450, family 71, subfamily A, polypeptide 13, which has been reported that can regulate camalexin levels and improve leaf expanding (Nafisi M. et al., 2007). Brara.F01137 is also the coding protein that belongs to the cytochrome P450 superfamily (cytochrome p450 79f1) which caused bushy phenotype in *Arabidopsis* (Birgit R. et al., 2001), and it was up-regulated only by Recipe M. The Brara.H01125 is also related to a cytochrome protein family. However, it was up regulated by Recipe M and R. Brara.H00687 was identified as a Rapid alkalinization factor (RALF) family protein and highly performed in Recipe M and B, which consists of extracellular signals and can

reduce root growth and development (Pearce G. et al., 2001). Brara.C04537 was identified as chloroplast beta-amylase 3 (BAM3), which was significantly high performed in Recipe B, followed by Recipe M. The BAM3 was reported by Kaplan F. et al. 2005 about its beta-amylase activity and cold stress response. Brara.G03530 refers to the NR gene that was down-regulated by Recipe M and up-regulated by Recipe B, while Brara.I00981 refers to NiR gene was down-regulated by Recipe M and Recipe R. These two genes have been confirmed to play a deciding role in plant nitrate metabolism (Lillo C. 1994).

Chapter 5

Discussion

Chapter 5. Discussion

5. 1 The newness that brought by urban agriculture

Compare with the traditional farming, the urban agriculture can deliver products that fit into a community’s particular standard, as a more sustainable ingredient provider. Stable is one of the most convincing points this agricultural mode is selling to the urbanized community. In particular, the vertical farm model is based on the full adoption of industrialized and digitally controllable equipment, which leads to the maximized artificial manipulation to the planting environment by reducing the impact from uncontrollable natural factors to product growth. This technical achievement would benefit agriculture products with stable quality as well as a steady and controlled yield. Meantime, the vertical farm model can reach the maximum capital-output ratio between natural resources consumption and products harvesting, avoiding superfluous natural resources abusing during the production process.

When it comes to the entire supply chain construction, efficacy and precession have become the priority for satisfying the urban catering industry's requirement. Urbanized new dining culture and food preferences are expanding rapidly with the intensity digitization, appearing to be e-commerce. This trend brings direct challenges to the restaurant business that the transport speed and supply chain streamlining become factors that improve merchants with more commercial value and net profit more than dine-In experience. Meanwhile, the stock room is designed to be more integrated with kitchens as well as the cooking spaces are developed to go public, which founds the potential marketing slot for vertical

farming. This advanced farming innovation can be established anywhere without being bound to natural resource. Similar to a mechanism composition or an independent kitchen facility, it could be built in the nearest place to the food processing counter, playing the role of food supply station of multiple merchants. Simultaneously, the big data system at backstage accomplishes the data collecting and analysing the information of all marketing movements, which helps to chase each progressing transaction and have timely insight into the city's general dining updates. As a consequence, that draws the city a more precise map of its food requirement. For vertical farming, it also relies on data analysing, aiming to reduce cost investment and taking more control of overall output by artificial techniques should be seen as a proper solution for meeting the city's future development.

However, while urban agriculture can produce more food with less natural resources and is far from harmful pesticides and fertilizers, it still faces the limitations of excessive energy consumption. Although its production model has brought about specific business opportunities for the change of supply chain and the demand of the food market, how to control energy consumption and output more accurately and improve revenue is the top priority for the further promotion of this technology. The birth of LED light has brought new solutions. Its stable, controllable and flexible features perfectly meet the needs of vertical agriculture, and its extremely high photoelectric conversion efficiency also dramatically helps to reduce energy consumption. Besides, its significant advantage is that its spectrum can be artificially controlled according to the photosynthetic characteristics, morphological establishment, quality and yield requirements of different plants, thereby further improving the stability of product quality. In order to achieve this goal, the current research needs are to conduct a comprehensive study of different types, different varieties, and crops of different growth stages to obtain a massive database of lighting methods. At the same time, the mechanism is investigated to summarize the foundation of

plant response to the spectrum, to expand the general use and ease of use of LED as an artificial light source. With the advancement and integrity of technology and the reduction of LED production costs, this new technology will be rapidly developed in industrial agriculture, family farming, urban agriculture and even space agriculture to fulfil people's more diverse demand for agricultural products.

5. 2 The optimizing of lighting recipes and physiological parameters of plant growth under LED spectra

Agricultural production is a highly complex field, and the massive number of crop varieties and the demands for product qualities have brought great difficulties to the adjustment of artificial conditions. Although the characteristics of LED lamps can perfectly meet the needs of urban agriculture, urban agriculture itself still need to compete with traditional agriculture, which has accumulated decades of industrial experience. This project focuses on optimizing LED lighting recipes by analyzing the physiological responses. The research started from two directions: the effects of the different spectrum and intensity, and the response from varied species and varieties.

5. 2. 1 Comparison of LED recipes between tomato, lettuce, and pak choi

Tomato is a crop with large cultivation area, enormous economic benefit and

rich nutrition deeply craved by the market. Compared with other common vertical farming crops, its more complex morphological structure and physiological changes such as flowering and fruiting bring more changing light conditions requirement. It has been reported that low-temperature and low-light intensity will have an inhibitory effect on tomato production, resulting in adverse effects such as weak plant growth, poor vegetative growth, falling flowers and reduced yield (Wang Y. L., et al., 2000; Han C., et al., 1990), but a suitable light quality will also promote its quality. Bird C. R. (1991) has shown that appropriate red-light treatment can increase the lycopene content in the fruit, as the expression level of phytochrome A is significantly increased under red light, which may be a regulator of carotenoid synthesis and related to the lycopene pathway. In comparison, the content of protein and vitamin C in tomato fruit was significantly promoted by blue light.

Although the overall developmental state of tomato has been suppressed to varying degrees under LED illumination, on the other hand, LED light sources exhibited a strong emphasis on plant morphology. As a crop with a more extensive form, tomato is often limited by space in actual production, especially in vertical farming where the application of space requires excellent efficiency, and the size of the plant needs to be precisely regulated. In the experiment, the plant height and leaf canopy of tomato were significantly regulated by blue light, which gave us more inspiration in actual production. On the other hand, lettuce and pak choi showed a complete growth cycle under an LED light source, and their yield was promoted to different degrees under changed LED recipes, especially pak choi showed a more positive response to LED light sources. In contrast, its excellent production yields and more energy savings reflect the outstanding potential of LEDs as a new generation of artificial light sources. Compared with previous studies, we have adopted a more exceptional light spectrum design based on the advancement of LED technology, which is not limited to integer ratios but more complex and diverse optical ratios. The results

also showed that the optimal LED recipes among different varieties indeed presented a slight difference in the red-blue ratio, which also brings us more space to explore for the light recipe database (Critten D. L. et al., 1993; Jao R. C. et al., 2003).

5. 2. 2 LED recipe on pak choi growth

Leafy vegetables are popular and commonly grown worldwide, especially the pak choi which widely planted in Southeast Asia. In traditional agriculture, pak choi had taken advantage of the market due to its low cost and rapid growth cycle. Thus, a completed LED lighting recipe should also approach similar results. For the experiment in both the UK and Malaysia campuses, the local varieties of each region all showed a positive response to the specific LED recipe. The red/blue: 70%/30% lighting method improved the yield and dry matter in both UK and Malaysia varieties. Due to the narrowband characteristic of LED wavelength, this recipe has been chosen from more precise intervals which giving it higher credibility. Whether considering long-term or short-term cultivation, red light is recommended as the primary spectrum required for plant growth. In addition to its photon type suitable for chlorophyll absorption, red light can also affect the broad-face orientation of chloroplasts through phytochrome, affecting light energy capture (Haupt W., 1965). Therefore, the spectrum dominated by red light is generally selected as the primary source of light (Bondada B. R. et al., 2003; Bukhov N. G. et al., 1992). It also has been observed that the ratio between red and blue light had a significant relationship with fresh matter / dry matter ratio, which associated with water content and water use efficiency.

The research of LED recipe on pak choi also involved the effect on different growth stages. The growth stage has been set to first 13 days after germination as early stage, and 14th day to 30th day as later growth stage. The fresh matter and dry matter showed a very similar tendency as the red/blue: 70%/30% performed the best yield, but the morphology results are more complicated and variety specific. However, many studies reported that blue spectrum may played an important role. Yun K. et al. (2018) suggested that blue spectrum treatment can promote elongation growth on petunia, calibrachoa, geranium, and marigold, as presented by an enhanced plant height, internode length, and stem extension rate. Saebo et al. (1995) reported that blue spectrum could improve leaf area and extend petiole, but it also leaded to the increasing of the activity to indoleacetic oxidase and reducing the level of auxin (IAA), thereby inhibiting apical dominance and achieving a bushy, much branched canopy.

Above that, with added far-red light, the pak choi flowering can be significantly brought forward. This phenotype was related to the response of phytochrome. In far-red light enriched conditions, P_R induces the dephosphorylation of Phytochrome Interacting Factor (PIF) proteins, which strengthens their ability to bind DNA and promote transcription of genes involved in shade-avoidance syndrome (SAS), including in the production of auxin and its receptors (Roig-Villanova et al., 2016). While Due to the flexibility of LED lights, the application of this phenomenon is more abundant. Yang Z. (2012) reported that the End-of-day methods, by adding far-red light at the end of each lighting cycle, the growth speed can be enhanced tremendously.

Meanwhile, the intensity of the LED recipe showed a strong relationship with energy use efficiency. During the experiment, once LED light intensity increased by 50%, the energy cost increased by 50% and the fresh matter also increased by 49.6%. This linear relationship showed a high photoelectric

conversion efficiency and gave us a more flexible way to set up the lighting conditions. The intensity between 130 μmol/m^2s to 150 μmol/m^2s will not stress pak choi growth and present similar energy use efficiency. As fluorescent lamp consumed more energy than LED lights, the energy use efficiency (yield per Kwh) of LED treatments has dramatically increased by 114%.

5. 2. 3 Diversity between different species and varieties

Although pak choi showed a positive response to the LED lighting recipes, the tomato seedling and lettuce performed differently, especially when the tomato seedling was stressed by major kinds of LED lighting, only specific red/blue ratio (73%/27%) brought the yield to the regular level. Meanwhile, the morphology was still undeveloped. Lettuce also showed negative results to most kinds of LED recipe, but with red/blue ratio at 73%27% the dry matter was slightly increased, compared with CK. It has been reported that red: blue at 80%/20% showed positive effects on lettuce growth. The net photosynthetic rate was enhanced while chlorophyll assimilation was reduced under this specific recipe, with its increase in the transpiration rate, stomatal conductance and intercellular CO_2 concentration of plants (Wen et al. 2009). Due to the limitation of experimental conditions, we are not able to explore more recipes for these species, but the results still showed us a more complicated spectrum requirement for tomato seedling growth, especially the formation of morphology.

Four Malaysian local pak choi varieties were chosen for the experiments. The final yield was enhanced by LED treatment, but the stem elongation and leaf development were drastically changed at an earlier growth stage. In the meantime, the chlorophyll synthesis was processed more regularly among the

varieties. Also, the relationship between leaf and root development was detected. It is crucial to notice that the specific variety Kungfu 2 performed the highest reading in root volume with LED treatment but lowest reading with fluorescent treatment. In the meantime, it acquired the highest leaf area with fluorescent treatment. It indicated that the carbon distribution was strongly affected in this variety as more biomass be transported for leaf expansion. Also, Green Hill 402 was repressed in root length and improved in diameter by LED treatments, which showed contrast results in fluorescent treatments. As blue light has been reported very related to root elongation and thickening (Yang Y. et al., 2010), and Yun K. highlighted the different sensitivity in elongation growth responses was linked to different shade-avoidance strategies between the different species (Yun K. 2018), it inspired us that the differences between pak choi varieties is most likely associated with the sensitivity to blue light, which regulated the morphology through carbon distribution.

Under the situation that the light condition is the limiting factor, the plant can reduce or enhance the ability of the leaf of capturing light energy by changing its leaf structure and chloroplast composition, consequently regulating the photosynthetic capacity of the plant leaves and realizing the carbon in different organs of the plant. When water and nutrients are sufficient, reasonable lighting conditions are beneficial to form more carbohydrates through photosynthesis and distribute them to the above-ground part, thereby causing an increase in the demand for nutrients and water absorbed by the roots, turn affects plant roots development. Therefore, in the condition of a fixed LED recipe, the pak choi during growth stages showed changed irradiation requirements. Thus, the carbon distribution also showed different responses between varieties (Groot C. C. et al., 2002; Kotowski W. et al., 2001).

5. 3 LED recipes effects on photosynthesis

During plant growth and development, light is both an energy source and a stimulus signal, while other environmental factors regulate plant growth and development by directly or indirectly affecting plant photosynthetic capacity (Ohyama K. et al., 2005). Previous studies have demonstrated the response of multiple varieties to different LED recipes, and many studies have revealed the effects of different monochromatic light on plant photosynthesis, physiology and biochemistry. Our experiments showed that the red-blue ratio of the composite LED recipes significantly formed the trend of continuous change in photosynthesis. In other words, the weight of spectra in the LED recipe is continuously changing. It is considered that understanding the red-blue ratio effects on the balance of plant photosynthesis is the key to understanding and quantifying plant photosynthetic efficiency.

5. 3. 1 The Effects of LED recipes on light stress

In many agriculture forms, light intensity is the most variable conditions, as it is affected by many factors and related to energy consumption in the vertical farming system. Thus, it is indispensable to explore the responses in the photosynthetic system. Our experiment about pak choi indicated that LED recipes as red/blue: 70%/30% had the highest carbon fixation rate under all light intensity, and CK had better performance than monochromatic light treatments. However, the responses in the PSII centre were more complicated, and it is necessary to note that with the lowest intensity, PSII still reaches higher

efficiency, and different treatment had very similar volumes. Unlike Pn results, PhiPS2 was increasing during 100-200 μmol/m^2s. This fact indicated that the status or activity of PS II is more important than the light source input for quantum yield, which located at the range of 100-200μmol/m^2s. Ilieva I. et al. (2010) also reported the lower LED intensity lead to higher PSII efficiency in lettuce, which considered that the low intensity would force the photosystem to open, to achieve the highest light capture capacity, and it is clear to submit that it also can be applied to varies LED spectrum. When intensity was increasing, the reduction in PhiPS2 demonstrated the self-protection mechanism, which had better performance in CK.

Significant improvement of blue light on stomatal movement has been observed. There have been many studies on the regulation of light quality on stomatal opening and closing. Zeiger E. et al. (1985) revealed the principle that blue light promotes stomatal opening. Blue light can activate plasma membrane, making ATPase continuously pumps protons to form a transmembrane electrochemical gradient. Consequently, it promotes the absorption of potassium ions by guard cells, resulting in decreased intracellular osmotic potential, water swelling of guard cells and opening the stoma. Meanwhile, Frechilla S. et al. (2000) found that blue light and green light play opposite roles in stomatal opening. They believed that this reversible reaction might be occurred by isomerization of zeaxanthin located on chloroplast, which refers to active form mainly absorbing green light, and the inactive form absorbing blue light, which is similar to the reversible reaction of phytochrome to red light and far-red light. In our experiments, all species treated by 100% blue LED light showed significant enhancement on stomatal opening than CK confirmed this hypothesis, as fluorescent lamp contained extra green light in the spectrum. It is also

mentioned that stomatal conductance was not changing after intensity reached 300 μmol/m^2s, which proposed that the ratio between each wavelength took the significant factors than the total quantum for stomatal movement.

5. 3. 2 The effects of LED lights on chlorophyll fluorescence

For UK local pak choi varieties (*Green revolution F1*), a similar tendency in qP and PhiPS2 was perceived. As qP indicated the reduction state of primary electron acceptor Q_A in PSII, this result showed that the electron flow from the PSII oxidation side to the PSII reaction center was not affected by the changed wavelength. Shunichi T. et al. (2010) reported that PSII was in the process of continuous damage and repair when it absorbed light, and it led to photoinhibition if the rate of damage is higher than repair. It was known that the oxygen-evolving complex of PSII is extremely sensitive to photodamage, which can be magnified by blue light. The results in Fv/Fm indicated that pak choi only required a certain amount of blue light to maintain PSII. It will not change after blue light proportion reached 30%.

It is also crucial to notice that the efficiency of quantum yield and the net photosynthetic rate was not linearly related under LED lighting, which usually showed a diverse response in outdoor growth. This result can be analyzed from two causations: first, the efficiency of carbon assimilation, significantly Calvin cycle was reduced by spectrum; second, the CO_2 became the limiting factor. It has been reported that different spectrum will affect the efficiency of Rubisco activity. Ernstsen J. et al. (1999) compared the Rubisco activity under different

light quality and indicated that green light could extremely reduce it, but no significant difference was observed between red and blue light. Meanwhile, the effects of blue on stomatal conductance were observed clearly in the experiments. Therefore, it has a high probability that the difference between LED recipes effects on photosynthesis is generated by the balance between PSII efficiency and CO_2 supply. The blue light played an essential role in PSII maintenance and CO_2 absorption by regulating guard cell opening.

5. 3. 3 Different photosynthesis performance between varieties

It is observed decidedly that, in the earlier stage the Pn had a weak difference between each variety but compared with CK, the LED treatments were increased. In the later stage, 802 Bushido and Kungfu 2 were higher than 702 Warrior and Green Hill 402 in Pn, and no difference between LED and CK treatments. As results, the yield of the four varieties combined the performance in Pn, as 802 Bushido and Kungfu 2 had greater yield than 702 Warrior and Green Hill 402, and each of them had better performance under LED treatments than CK. It is vital to notice that the increasing yield between LED and CK is same as the previous experiment in UK varieties, which may suggest that this red/blue: 70%/30 is highly versatile for pak choi varieties. Nevertheless, the biomass assimilation in 802 Bushido is 12.5% higher than Green Hill 402, which showed sensitivity between varieties for lighting conditions.

It has been discussed before about the turbulent growth status in different

growth stages of pak choi in chlorophyll synthesis and morphology, and the same tendency also expressed in the contemporaneous stomatal movement. The stomatal conductance was deeply reduced in the later stage. Compared with PhiPS2 and Pn, the PhiPS2 showed no difference in both of the two stages while Pn was reduced. It was identified that for long term growth, the CO_2 supply became the limiting factor for pak choi growth as the guard cell was ageing and decay. It is more evident in LED treatments, and CK showed more resistance. 802 Bushido remained relatively higher stomatal conductance in later stages, leading to higher water consumption.

5. 4 Insight and exploratory of RNA-sequencing

The High-throughput sequencing technique is a revolutionary advance to traditional sequencing. This technology which can sequence millions of DNA molecules at once and enables detailed analysis of the transcriptome and genome of species. It is possible to perform sequencing at the transcriptome level, also known as RNA-Sequencing (RNA-Seq), which is the starting point for studying gene function studies. Thus, the study of alternative splicing regulation, transcript variation or non-coding RNA expression can be carried out in successfully (Wei T. et al., 2016). RNA-Seq can provide accurate digital signals, with the advantages of fast sequencing speed, improved accuracy and low cost compared with traditional sequencing methods. RNA-Seq results are detected with known genomic DNA sequence information to judge gene expression, which can be applied to explore gene expression, optimise the gene structure and discover new genes. It is a powerful tool for the in-depth study of complex transcriptomes (Margulies A. 2005).

This study performed RNA-seq on pak choi under different LED light treatments, and the results, comparing the results with the *Brassica Rapa* genome（BrapaFPsc_277）. The objects were grown under LED lighting, by analyzing the differences in gene expression of the whole pak choi genome, the response has been synoptically understood, especially concerning the genes involved in light-harvesting and photoreactions. The results clearly showed the dramatic changes in plant photoreactions under LED light sources, especially in the light-harvesting protein complex, where several damage and repair phenomena occurred. The high expression of several proteins associated with photosystem repair tells us about the damage to the photosystem. Compared with the previous chlorophyll fluorescence measurements, it is apparent that LED light had particular stress on the operation of the photosystem. However, since the photon energy carried by the LED spectrum was very suitable for photoelectric reaction, its electron output still showed a positive result. While blue light is also involved in the regulating photosynthetic system repair, carbon and nitrogen cycle, stomatal movement, and other vital mechanisms, under this joint action, plants still showed improved efficiency of light energy utilization (Yu R. C. 2002).

5. 4. 1 The damage and repair of LED light in pak choi photosystem

As the most important abundant light energy captures and transports organs, the light-harvesting complexes (LHC) in photosystem II and I are most sensitive to lighting conditions. The RNA sequencing results indicated that most kinds of LHC were downregulated by combined LED recipe. Even though the suppression was not significant, it still showed the shortcoming of the monotonous spectrum. However, the early light-induced proteins (ELIP) family

showed a positive reaction to LED lighting, as it was up-regulated by combined LED recipe and extremely highly performed in blue light. It has been reported that individual red light, far-red light and blue light can induce ELIP expression, and blue light induces more ELIP than red light (Adamska I. et al., 1992). The *ELIP* gene has three transmembrane regions, and the amino acid sequences of these three transmembrane regions are highly similar to the corresponding regions of chlorophyll a/b binding protein. Both can bind to chlorophyll and carotenoids (Heddad M. and Adamska I., 2000), so the ELIP protein is classified into the chlorophyll a/b binding protein superfamily. Furthermore, this structure is highly conserved among different species, suggesting the importance of such structures in functioning (Heddad M. and Adamska I., 2000).

There have been many reports on the function of ELIP. Hutin C. (2003) studied the *chaos* mutant of *Arabidopsis thaliana* and found that this mutant could not accumulate ELIP rapidly and the plant was not resistant to intense light, which made ELIP widely believed to have photoprotective effect. Under a photoinhibition conditions, the accumulation of ELIP is accompanied by a decrease in LHCII mRNA levels and a rapid replacement process of the D1 protein in the PSII centre, indicating that it may be involved in the repair process of damaged photosynthetic organs (Wierstra and Kloppstech 2000), while ELIP can also reduce free chlorophyll accumulation to avoid photooxidative stress. As a result, ELIP was involved in photosynthetic response with LHC protein to repair the damage that caused by LED spectrum and maintain the function of photosystem at an average level (Figure 5. 4. 1).

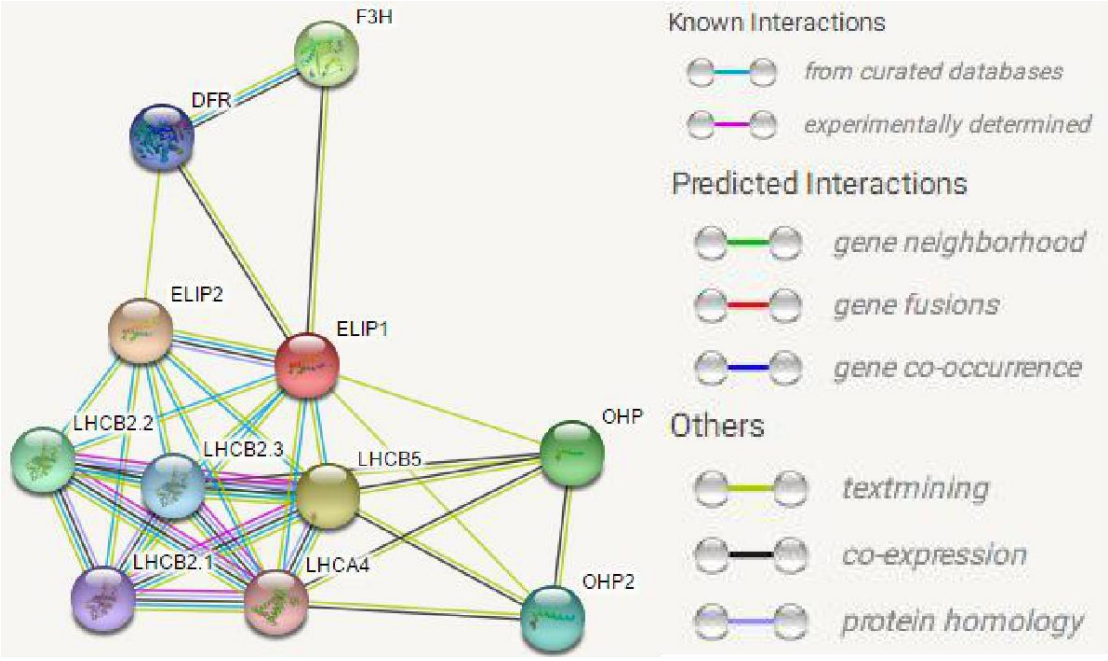


Figure 5. 4. 1 Network of light-harvsting complex gene. Source: String network.

5. 4. 2 Morphology and quality related genes

At the same time, other genes that related to morphology and plant quality and content were also detected. Brara.H01691 is related to H^+-ATPase 1, as the enzyme is acting on acid anhydrides to catalyse a transmembrane movement of substances. It is wildly used for secondary transport, which is involved in many physiological developments such as solute uptake in roots, tip-growing systems, size of stomatal aperture and cell wall extending (Gévaudant F. 2007; Auer M. 1998; Yanovsky M. 1995). As it was highly performed in monochromic blue light, it is confirmed with previous results that the blue light has positive effects on stomatal conductance and hypocotyl elongation. In the meantime,

Brara.H00687 by regulating RALF family protein to arrest root growth. The high performance in Recipe M indicated that LED lights would inhibit root development. However, the results in root scanning in Chapter 3 showed that in four tested pak choi varieties, only two of them had limited root growth, which may indicate that this differently expressed gene was variety specific. Brara.F01137, which coded cytochrome family protein, had no significant expression in Recipe R and B but up-regulated by Recipe M, and it has been reported that in *Arabidopsis*, CYP79F1 can increase the concentrations of indole-3-acetic acid and indole-3-acetonitrile, which related to glucosinolates mediation. It had essential effects on plant leaf development (Birgit R. 2001). Meanwhile, CYP706A1 (cytochrome P450, family 706, subfamily A, polypeptide 1) regulated by Brara.H01125 showed the function of positive improvement in the biosynthesis of both short- and long-chain aliphatic glucosinolates, located in seeds, rosette leaves, stems and cotyledons of Arabisdopsis. The aliphatic glucosinolates have a notable role in plants flavor, nutritional value and pest resistance, which is also crucial in pak choi growth and commercial value (Chen S. et al., 2003). As Brara.C01573 has been reported by Nafisi et al., 2007 that can regulate camalexin level in *Arabidopsis*, which is vital for resistance to necrotrophic fungal pathogens. Brara.C04537 can mediate the accumulation of maltose in *Arabidopsis*. Although it usually responds to cold stress, it in some way showed protection to the photosynthetic electron transport chain (Kaplan F. 2005).

In the meantime, the NR and NiR gene expression, which related to nitrite metabolism, was also regulated by LED recipes. NR was up-regulated by blue light while NiR was down-regulated by red light, and both of them were down-

regulated by Recipe M. In plant growth, NO_3^- is first reduced to NO_2^- under the catalysis of NR, in order to prevent the toxic of NO_2^- to plant the NO_2^- will be immediately reduced to NH_4^+ by the catalysis of NiR (Rongchen W. et al., 2003). Thus, the NR and NiR are both positively related to nitrite reduction. Although Recipe M had adverse effects on NR and NiR expression, the monochrome red or blue light showed different effects, indicating some other lighting methods to regulate plants quality.

Chapter 6

Conclusion

Chapter 6. Conclusion

6. 1 Conclusion

The born of urban farming aims to seek an alternative approach to solving the global food crisis, under the pressure of climate-changing and natural resource collapsing. Ulteriorly, this agriculture form also existing as the industrial outcome which contributed by the iterative technology innovations, a range of environmental regulation methods, including LED lights, makes this resource-efficient use of growth pattern getting more opportunities and offers new possibilities in the areas of urban food supply, richer diet, and environmental protection.

In our studies, the LED red/blue ratio at 70% to 30% with 130~150μmol/m^2s intensity has become the most suitable recipe for pak choi biomass targeting growth, and it can apply to different growth stages and some specific varieties. Meanwhile, the different LED recipes have specific effects on pak choi morphology, such as plant height, leaf area, canopy construction, and shoot-root ratio, especially in their early-stage growth, indicating diverse regulation methods for plants growth.

Above that, for photosynthesis among pak choi varieties, the light reaction was strongly regulated by LED recipes, while the quantum yield and electronic conversion efficiency were less affected. Red light played the most crucial role in dry matter accumulation, while blue light was functioning in photosynthetic

system repair and maintenance. In the meantime, the stomatal movement was mainly affected by blue light, and it showed different sensitivity between each variety, which related to the CO_2 absorption and transpiration. The red and blue spectrum ratio in the LED recipe has mainly affected the efficiency of the photosynthetic reaction center and utilization of CO_2. Thus, the intercellular CO_2 concentration was considered to evaluate or even forecast the effects of LED recipes. Combined with the variety difference, this finding may provide a new way to set up new LED recipes for unknown species or variety and affects the overall output and resource use efficiency.

The RNA-sequencing results demonstrated the insight response in pak choi against specific LED lighting conditions, especially the stress and repair in the photosystem. In the meantime, other essential genes related to plant development, stress tolerance and metabolite synthesis were identified. However, due to the funding and time constraints, it is not able for more replicates and better-quality samples for testing. Also, the results of RNA-seq were not verified by qRT-PCR, which harmed the correctness of the results. Nevertheless, it is undeniable that the experiment still gives us a quantitative analysis of gene expression and detection of photosynthetic pathways and presented us with a deeper understanding of the specific effects of LED light sources.

6. 2 Limitations

Although we have obtained some informative and prospective results in this project, our experiments were limited by many force majeure factors. As we were the first group that focus on the effects of LED lights on plants growth, we

had to set up the experimental equipment from the very beginning, especially the selection, procurement, and installation of LED facilities. As our project was conducted in two campus, it doubles the time and resources we spend on preparation and affected the experimental design. Meanwhile, some unexpected accidents had also delayed the progress, such as the fault of air conditioner in growth room.

In general, there are few improvements we should achieve with better project planning: (1) The differences between varieties should also be detected by RNA sequencing, to investigate the changed gene expression between varieties. (2) The identified genes should be confirmed through real-time PCR analysis, that can make the results more accurate and justified. (3) The nutrition measurements should be involved, which can supply more information about the value of vertical farming production.

6. 3 Future work

In commercial agricultural production, the study of the red-blue ratio function is particularly critical. Since plant photosynthesis and organ development are an extremely complex dynamic process, simply studying the functions of a specific wavelength cannot cope with the complex needs of the actual implementation. At present, the research focus is mainly on the exploration of LED light application and the study of photosynthesis mechanism. When the data collection achieves a certain point, the author believes that we should focus on model construction and integrate all aspects of data from a dynamic perspective. Revealing the relationship between various regulatory factors has a more direct guiding effect on meeting the production requirements, which will bring truly

vitality to this technology.

1. Research on LEDs relies heavily on the quality of the light source itself, especially its spectral stability and dispersion, as well as the distribution density of the light source. Its characteristics will affect the growth of plants to a certain extent, especially compared with other artificial light sources. In the future, it is recommended to test broader types of LED lights to provide a more detailed evaluation of artificial light sources.

2. Because the photosynthetic system of plants is a highly complicated structure, and the influence of radiation on it results from multiple aspects, mathematical models are proposed in future research to systematically study the effects of multiple influencing factors on overall photosynthesis.

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Appendix 1. RNA extraction

Preparation

A. Required reagents:

Chloroform and Isopropyl alcohol, analytically pure;

75%, 80% and 100% Ethanol;

RNease-free water

TRIzol reagent;

B. Required equipment:

Micropipettors;

Vortex mixer;

Refrigerated centrifuge;

Incubater;

RNA isolation

1) Grand the tissue with liquid nitrogen, carefully collect the tissue into centrifuge tube, then add TRIzol for 1 ml per 100mg tissue.

2) Centrifuge the samples with 13,000 xg for 15mins, at the tempreture of 2 to 8 degree.

3) Transfer the supernatant, add 200μl chloroform (per 1 ml TRIzol used).

4) Shake the tubes for 15s on the mixer, incubate it at 30 degree for 15mins;

5) Centrifuge the samples with 12,000xg for 15mins, at the tempreture of 2 to 8 degree.

6) Transfer top aqueous to new centrifuge tube, then repeat from step 3);

7) After repeating step 3), add 500 μl isopropyl alcohol per 1ml TRIzol;

8) Incubate the sample at 30 degree for 10mins, centrifuge at 12,000xg for 10mins at 5 degree.

9) Remove the supernatant carefully with micropipettors, wash the RNA pellet with 1 ml 75% ethanol per 1 ml TRIzol, centrifuge at 7,500 xg for 5mins at 5 degree.

10) Air dry the RNA pellet. Dissolve in 50μl RNease-free water, shaked with mixer for 5 mins, then incubate it for 10mins at 60 degree.

DNA clean up

1) Mix 2.5μl DNase I stock solution with 10 μl RDD buffer;

2) Add the mixed reagent to RNA solution, incubate it for 15 mins at 30 degree;

RNA clean up

1) Adjust the RNA solution to 100μl with RNase-free water, add 350μl RLT buffer;

2) Add 250μl 100% ethanol, shake the samlpe by mixer for 5mins.

3) Apply 700 μl sample to RNeasy MinElute Spin Column, centrifuge it for 15s at 8,000xg, discards the flow-through;

4) Transfer the spin column into new 2ml tube, add 500μl buffer RPE, centrifuge for 15s at 8,000 xg, discards the flow-through.

5) Add 500μl 80% ethanol, centrifuge for 2mins at 8,000xg;

6) Transfer the column into a new 2ml tube, open the cap and directly put into centrifuge for full speed spin for 5 mins.

7) Transfer the column to a new 1.5ml tube, add 14μl RNase free water, centrifuge at full speed for 1 min.

Appendix 2. Annotations of selected genes in Table 4. 3. 2

Feature ID	Gene symbol	Annotations
Brara.B01738	PSBY, YCF32	photosystem II BY
Brara.A00858	PSB28	photosystem II reaction center PSB28 protein
Brara.A01166	PSBQ, PSBQ-1,PSBQA	photosystem II subunit QA
Brara.J00193	PSB27	photosystem II family protein
Brara.H00652	PSBX	photosystem II subunit X
Brara.F03187	LHCB2, LHCB2.3,LHCB2.4	photosystem II light harvesting complex gene 2.3
Brara.F00021	PSAG	photosystem I subunit G
Brara.H02525	CP24, LHCB6	light harvesting complex photosystem II subunit 6
Brara.I03747	LHCA1	photosystem I light harvesting complex gene 1
Brara.G00547	PSAF	photosystem I subunit F
Brara.H02384	LHCA2*1, LHCA6	photosystem I light harvesting complex gene 6
Brara.C04035	LHCB2, LHCB2.1	photosystem II light harvesting complex gene 2.1
Brara.B03307	LHCB2, LHCB2.3,LHCB2.4	photosystem II light harvesting complex gene 2.3
Brara.I00765	PSAN	photosystem I reaction center subunit PSI-N, chloroplast, putative / PSI-N, putative (PSAN)
Brara.E02107	PSBTN	photosystem II subunit T
Brara.I02857	AB140, CAB1,CAB140,LHCB1.3	chlorophyll A/B binding protein 1
Brara.I02817	PSAK	photosystem I subunit K
Brara.C03812	PSBTN	photosystem II subunit T
Brara.A00845	PSAE-2	photosystem I subunit E-2
Brara.I04910	CP24, LHCB6	light harvesting complex photosystem II subunit 6
Brara.J00467	PSBW	photosystem II reaction center W
Brara.G00467	PSBX	photosystem II subunit X

Brara.E03123	LHCB4.2	light harvesting complex photosystem II
Brara.F01095	CP24, LHCB6	light harvesting complex photosystem II subunit 6
Brara.D02090	AB140, CAB1,CAB140,LHCB1.3	chlorophyll A/B binding protein 1
Brara.A02793	PSBTN	photosystem II subunit T
Brara.H01891	PSAK	photosystem I subunit K
Brara.F00256		Photosystem II 5 kD protein
Brara.I04677	PSB29, THF1	photosystem II reaction center PSB29 protein
Brara.F02286	PSAN	photosystem I reaction center subunit PSI-N, chloroplast, putative / PSI-N, putative (PSAN)
Brara.E00101	PSAP, PSI-P, PTAC8, TMP14	photosystem I P subunit
Brara.H02952	OE23, OEE2, PSBP-1, PSII-P	photosystem II subunit P-1
Brara.E01452	PSAG	photosystem I subunit G
Brara.I00297	LHCB2, LHCB2.3, LHCB2.4	photosystem II light harvesting complex gene 2.3
Brara.H00553	PSAL	photosystem I subunit l
Brara.E01277	PSBW	photosystem II reaction center W
Brara.J00907	LHCB3, LHCB3*1	light-harvesting chlorophyll B-binding protein 3
Brara.D02820	PSAP, PSI-P, PTAC8,TMP14	photosystem I P subunit
Brara.F00523	PSAO	photosystem I subunit O
Brara.D01828	PSBW	photosystem II reaction center W
Brara.B00018	LHCB4.2	light harvesting complex photosystem II
Brara.G01647	LHCA1	photosystem I light harvesting complex gene 1
Brara.H00039	PSAG	photosystem I subunit G
Brara.H00722	PSAF	photosystem I subunit F
Brara.C02638	LHCB5	light harvesting complex of photosystem II 5
Brara.I02744	PSAF	photosystem I subunit F
Brara.H01465	PSAE-2	photosystem I subunit E-2
Brara.I05310	PSAO	photosystem I subunit O

Brara.A02541	LHCA3	photosystem I light harvesting complex gene 3
Brara.I02349	LHCB5	light harvesting complex of photosystem II 5
Brara.D00751	PSAL	photosystem I subunit l
Brara.E00573	LHCB4.3	light harvesting complex photosystem II
Brara.F00205	PSAH-2, PSAH2, PSI-H	photosystem I subunit H2
Brara.B03997	MSP-1, OE33, OEE1,OEE33,PSBO -1,PSBO1	PS II oxygen-evolving complex 1
Brara.H00177	PSAH-2, PSAH2,PSI-H	photosystem I subunit H2
Brara.E01976	ELIP, ELIP1	Chlorophyll A-B binding family protein