Improvement of the growth, quality and fertilizer use efficiency of basil by regulating the environmental conditions

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（ 千葉大学審査学位論文）

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ABSTRACT OF THE THESIS

More and more producers are beginning to grow basil (*Ocimum basilicum* L.) in green house and plant factory with artificial lighting (PFAL), due to its multi-purpose use. In PFAL, light intensity, light spectrum, photoperiod, nutrient solution (NS), carbon dioxide concentration, temperature and humidity can be adjusted according to different purpose. The growth, secondary metabolite accumulation and nitrate content of basil are significantly affected by environmental conditions. Therefore, PFAL have been increasingly used to control the growth and secondary metabolite accumulation in basil to produce high-yield and high-quality basil. On the other hand, NS contains a lot of nutrients. The discharged NS results in a waste of nutrients and environmental pollution. The absorption and utilization of nutrients in the NS can be optimized by controlling NS management methods and light conditions. Therefore, the waste of resources due to the discard of the NS can be reduced by regulating the NS management methods and light conditions in PFAL.

The purpose of this study was to improve the growth, quality and fertilizer use efficiency of basil by modulating the light spectrum and NS management methods. This thesis consists of 5 chapters and the contents of each chapter are as follows.

In chapter 1, a general introduction including the overview of basil, some studies on basil, NS management methods, light conditions in PFAL, and the purpose of this research are introduced.

In chapter 2, the effects of NSs with different electrical conductivity (EC) values and different treatment days on the yield, total phenolic content (TPC), and antioxidant capacity of basil were studied. There are two experiments in this study. In experiment 1, basil plants were treated with four different ECs after transplanting and the treatment days were 18 days. The results in experiment 1 showed that the shoot fresh and dry weights, leaf fresh and dry weights, and leaf area were highest at an EC of 3.0 dS m^{-1} as compared to other ECs. However, the TPC and antioxidant capacities of basil were significantly promoted by low ECs (0.5 and 1.0 dS m^{-1}) as compared to

high ECs (3.0 and 5.0 dS m^{-1}). In experiment 2, basil plants were grown for 13 or 15 days in a NS with an EC of 3.0 dS m^{-1} and then treated with water or NSs with ECs of 0.5 or 1.0 dS m^{-1} for 3 or 5 days. The results in experiment 2 showed that the TPC and antioxidant capacity in basil leaves were significantly improved by short-term low-EC-treatments without significantly reducing the basil yield, compared to the control.

In chapter 3, the effects of different applied nutrient quantity (ANQ) in NS and red:blue (R:B) ratios on the growth and fertilizer utilization of basil were studied. Basil plants were treated with 4 ANQ treatments and three R:B ratios from transplanting to harvest (20 days). Results showed that low ANQ significantly improved the nutrient use efficiency and nutrient absorption efficiency. Furthermore, the yield of the basil plant and the absorption of N and K were significantly influenced by different R:B ratios under low ANQ treatments. Therefore, this study has determined the optimal combination of the ANQ and R:B ratio for improving the growth, nutrient use efficiency, and nutrient absorption efficiency of basil plants. The findings of this study can be applied to hydroponic basil production for saving resources and protecting the environment.

In chapter 4, the effects of different ANQ in NS and R:B ratios on the growth, secondary metabolite accumulation and nitrate content of basil plants were investigated. Basil plants were treated with three ANQ (0.5, 1 and 2 times (T) of the absorption quantity of nutrients determined in the preliminary experiment, indicated by 0.5T, 1T, 2T and 4T respectively) and three R:B ratios (3:7, 7:3 and 9:1, indicated by RB3:7, RB7:3 and RB9:1). The results showed that the leaf fresh and dry weights were not significantly affected by different ANQ treatments, however, the ANQ of 0.5T significantly improved the antioxidant capacity and TPC as compared to other ANQ treatments, regardless of R:B ratios. On the other hand, compared with other R:B ratios, the yield and accumulation of secondary metabolites of basil were significantly increased by RB7:3 under the ANQ of 0.5T. Moreover, the nitrate content significantly decreased with the decrease of ANQ, regardless of the R:B ratios. Therefore, the combination of the RB7:3 and ANQ of 0.5T is optimal for improving the yield and quality of basil in the present study.

In chapter 5, the results of this study are summarized. Moreover, proposals for future work to investigate the effects of light intensity and photoperiod combined with quantitative nutrient management method on basil growth and nutrient utilization were provided.

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LIST OF ABBREVIATIONS

ANQ: applied nutrient quantity

Chl a: chlorophyll a

Chl b: chlorophyll b

DPPH: 1,1-Diphenyl-2-picrylhydrazyl

DMF: N,N-Dimethylformamide

EC: electrical conductivity

ETR: electron transport rate

F_m: maximum chlorophyll fluorescence yield

F_o: minimum chlorophyll fluorescence yield

Fs: steady state chlorophyll fluorescence level

F'_o: minimum chlorophyll fluorescence yield

F'_m: maximum chlorophyll fluorescence yield

Gs: stomatal conductance

GAE: gallic acid equivalents

LEDs: light emitting diodes

LMA: leaf mass per area

NS: nutrient solution

NA: nutrient absorption

NUE: nutrient use efficiency

NAE: nutrient absorption efficiency

NW: nutrient waste

PPFD: photosynthetic photon flux density

PFALs: plant factories with artificial lighting

P_n: net photosynthetic rates

PhiPSII: quantum yield of PSII electron transport

QNM: quantitative nutrient management

qP: photochemical quenching

qN: non-photochemical quenching

R:B ratio: red:blue ratio

TE: trolox equivalents

TPC: total phenolic content

Tr: transpiration rate

TAN: total amount of applied nutrients

WN: final amount of remaining nutrients

WUE_{Pn}: water use efficiency of photosynthesis

CHAPTER 1. General Introduction

1.1. Overview of basil

1.1.1.Classification

Kingdom: Plantae

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: Ocimum basilicum L.

English name: Basil

Japanese name: バジル

1.1.2. The botany

Figure 1.1. The morphological characteristics of basil plants (A, B).

Basil plants are typically 18 to 24 inches tall and have blue, purple, and white petals. The leaf type is lobed or non-lobed but not separated into leaflets, and leaves arranged with two leaves per node along the stem. There are at least 65 species of basil and the morphological characteristics of different species are different (Makri and Kintzios, 2008).

1.1.3. Growth and development conditions

Most basil species come from Asia, Africa and South and Central America, and Africa is the main source of species (Paton and Putievsky, 1996). Basil is mainly propagated by seeds, and its suitable temperature and soil pH range for growth is 7-27 ℃ and 4.3-8.2, respectively. The seeds of basil are small and sown at a depth of 3-6mm, and the seeds germinate at about 8-14 days. To promote side shoots growth, the top of basil can be removed when it is about 15 cm tall (Makri and Kintzios, 2008).

1.1.4. The uses

Medicinal and pharmacological properties

Basil is rich in antioxidants (Filip et al., 2016), and phenolic compounds are an important part of antioxidants in plants (Velioglu et al., 1998). The mechanisms of the antioxidant activities of phenolic compounds are different, mainly including scavenging free radical, donating hydrogen, singlet oxygen quenching, metal ion chelation and act as substrates (superoxide and hydroxyl) for radicals. Moreover, it was reported that the antioxidant capacity is well related to the total phenolic content (TPC) in plants (Kaur and Kapoor, 2002). Therefore, the antioxidant capacity can be increased by improving the TPC. Rosmarinic acid and chicoric acid are the two most abundant phenolic compounds in basil (Lee and Scagel, 2013; Zgórka and Głowniak, 2001). There are many functions of rosmarinic acid, such as antibacterial, anti-inflammatory, antiviral, against pathogens and herbivores, act as UV-protectants etc. (Petersen, 2013). Besides, basil is used for pharmaceutical and cosmetic preparations due to it is rich in rosmarinic acid (Kiferle et al., 2011). Chicoric acid is the second principle phenolic compounds in basil. Chicoic acid not only protects plants from being injured by bacteria and pests, but also has the properties of anti-cancer, anti-viral, anti-diabetic and anti-obesity (Lee and Scagel, 2013). In addition, basil contains a variety of essential oils with medicinal value, which makes basil an important crop for producing essential oils (Hanif et al., 2011).

Culinary utility

Fresh or dried basil leaves can be used to add a unique aroma and flavor to foods. Moreover, they are also used in the production of cheese, beverages, teas, beverages, and vinegar, etc., and the essential oil extracted from basil flowers and leaves is also used in food processing. Additionally, basil seeds are rich in protein (11.4—22.5 g/100 g), dietary fiber, minerals (Ca, K and Mg) and phenolic compounds (orientine, vicentine, and rosmarinic acid). Therefore, basil seeds are considered a functional food and source of functional ingredients added to foods in recent years (Calderón et al., 2021).

1.2. Literature review: Effects of NS and light conditions on basil plant

There are many studies on the effects of NS and light conditions on basil growth and secondary metabolite accumulation. Among these studies, some were on the effect of electrical conductivity (EC) of NS on the growth and secondary metabolite accumulation of basil. For example, it is reported that different EC values did not affect the growth of basil plants (Walters and Currey, 2018) and the EC value of 2.8 dS m^{-1} significantly promoted basil growth (Morano et al., 2017); The fresh weight of basil plants was significantly improved by the EC of 1.0 dS m^{-1} as compared to other ECs (Ciriello et al., 2020). Different ECs (ECs of 1.2, 4.9 and 8.6 dS m^{-1}) did not affect the TPC of basils (Bekhradi et al., 2015); The EC of 3.0 dS m^{-1} significantly enhanced the TPC in basil as compared to the EC of 1.0 dS m^{-1}, however, for another cultivar there was no significant difference of the TPC among different ECs (Ciriello et al., 2020). The EC of 4.0 dS m^{-1} significantly improved the antioxidant activity in basil as compared to other ECs (Maggio et al., 2006). In addition, there are also some studies showed that low in one or several nutrients in the NS has a significant effect on the accumulation of secondary metabolites. For instance, a NS with low N content significantly promoted the accumulation of phenolic compounds in basil (Kiferle et al., 2013) and the content of antioxidants and phenolic compounds were significantly improved by nutrient limitation (Jakovljević et al., 2019). From these studies, On the other hand, there are some studies on the effect of different R:B ratios on the growth of basil. For example, one study reported that the shoot fresh weight of basil reached its maximum at R:B ratio of 7:10 (RB7:10) (among RB7:10, RB1:1, RB3:2, RB6:1) (Piovene et al., 2015). However, in other studies, the shoot fresh weight of basil reached its maximum at RB3:1 (among RB1:2, RB1:1, RB2:1, RB3:1, RB4:1) (Pennisi et al., 2019) and RB4:1 (among RB4:1, RB2:1, RB1:1) (Lin et al., 2021). The shoot fresh weight of basil were not affected by increasing the proportion of blue light from 20％ to 60％ (Larsen et al., 2020) and 0 to 100％ (Schwend et al., 2016). Moreover, the effect of light intensity on the basil growth were also studied. The plant fresh weight was increased by increasing the photosynthetic photon flux density (PPFD) from 100 to 300 μmol m^{-2} s^{-1} (Larsen et al., 2020) and the shoot fresh and dry weights increased significantly as PPFD was increased from 100 to 250 μmol m^{-2} μmol m^{-2} s^{-1} and any further increase in PPFD produced no further changes in these parameters (Pennisi et al., 2020). However, there were few studies on the effect of light conditions combined with NS on the basil growth. Therefore, exploring the effects of light conditions combined with NS on the growth of basil is of great significance for optimizing the cultivation conditions of basil from the aspects of light regimes and nutrient solution management.

From the above studies we know that the growth and secondary metabolite accumulation are significantly affected by the nutrient content and light conditions. Therefore, improving the yield and quality of basil by controlling the NS and light conditions in PFAL can be fulfilled.

1.3. Plant factories with artificial lighting (PFALs)

PFALs, also called indoor farm, is a closed system used to produce valuable plants such as vegetables and herbs (Pennisi et al., 2019). Moreover, the environmental conditions (NS, light, CO_2 concentration and temperature etc.) in PFALs can be controlled (Kozai 2018). The yield and quality in vegetables and herbs have been greatly improved by controlling these environmental conditions (Pennisi et al., 2019, Dou et al., 2017, Piovene et al., 2015, Song et al., 2020). As compared to traditional agriculture, PFALs improve the nutrient, land and water use effeciency (Graamans et al., 2018). With the global population increases and the available arable land decreases, PFAL is bound to become more and more popular due to it is not dependent on arable land and that can be established also in the urban (Kalantari et al., 2017). In countries such as Japan, China, America, etc., PFALs have been being used to produce leafy greens, herbs, medicinal plants, and other vegetables (Kozai 2018).

1.4. NS management methods in PFAL

In PFAL, hydroponics is the main method of vegetable cultivation. The EC value of NS is easy to be monitored and controlled. Moreover, the EC has important influences on the yield and quality of vegetables and herbs. Therefore, the EC management method of NS is a commonly used method in hydroponics (SharathKumar et al., 2020). However, the EC management method also has its disadvantages. For instance, although the the total amount of available nutrients in the NS can be reflected by the EC value of the NS, a specific nutrient content cannot be reflected (Miller et al., 2020). Additionally, vegetables often absorb large amounts of nutrients due to the rapid absorption of some nutrients (e.g., NO_3^-, PO_4^- and K^+) under the NS of the optimal EC. However, when the absorption of these ions exceeds a threshold (luxury absorption), the yield of the plant will no longer increase (Maneejantra et al., 2016), which will result in the waste of resource. Moreover, the luxury absorption of NO_3^- and K^+ result in the high concentration of them in vegetables and eating vegetables high in potassium ions can cause human diseases (Sago and Shigemura, 2018; Bikbov et al., 2020). Moreover, $Ca^{2+}$and Mg^{2+} will be accumulated in the NS due to the weak absorption capacity of these two ions by plants and Na^+ will also accumulate in EC-regulated NS. Therefore, even if the EC value of the NS reaches the target value, the concentration of the main nutrients required by the plant may still be sub-optimal. This is because the accumulation of Ca^{2+}, Mg^{2+}, HCO_3^- and Na^+ masks the deficiency of other nutrients. Therefore, NS controlled by regulating EC value for a long time may results in crop yield reduction and many planters prefer to discard the NS after harvesting (Miller et al., 2020).

The discharge of nutrients into the environment will cause a series of environmental problems, such as eutrophication of surface waters and coastal marine ecosystems, nitrate pollution of groundwater, the increase of the greenhouse gas concentration (nitrous oxide) and the development of photochemical smog etc. (Schwend et al., 2016; Larsen et al., 2020; Pennisi et al., 2020). Specifically, the discharge of a large amount of PO_4^{3-} into the water body leads to the eutrophication of the water body (Kumar et al., 2016; Vats et al., 2005). The nitrogen fertilizer in the NS mainly exists in the form of NO_3^- and NH_4^+ and the NH_4^+ is converted into NO_3^- by the nitrification of microorganisms in soil. These NO_3^- will enter the groundwater and cause excessive nitrate concentration. Too high NO_3^- concentration in drinking water is harmful to the human body (Savci, 2012). K^+ is an important source of groundwater salinization in semi-arid context (Buvaneshwari et al., 2020). Na^+ may cause soil particles to be dispersed and soil nutrient deficiency or imbalance, thereby affecting plant growth (Machado et al., 2017).

The quantitative nutrient management (QNM) is a novel NS management method in which nutrients are regularly and quantitatively added to the nutrient solution regardless of the nutrient concentration (Maneejantra et al., 2016). Although the nutrient concentration will be low and fluctuate by using this management method, the nutrients can also be absorbed well by vegetables (Sago and Shigemura, 2018). Moreover, QNM makes it possible to avoid luxury absorption of nutrients and increase the nutrient utilization by plants without affecting plant growth and wasting resources (Maneejantra et al., 2016) due to the nutrients can be supplemented according to the nutrient requirements of vegetables. In summary, compared with the EC management method, the QNM is more beneficial to the future development of hydroponics in terms of saving resources and regulating vegetable quality. QNM has been used to control the growth and quality of vegetables due to its advantages in saving resources in recent years.

1.5. Effect of light condition on the growth and phytonutrients of basil in PFAL

The growth of vegetables and herbs are significantly affected by light quality (Dou et al., 2017; Pinho et al., 2017; Piovene et al., 2015; Pennisi et al., 2019). In the spectral range of photosynthetically active radiation (400–800 nm), the quantum yield curve has two peaks in the range of red light (600–700 nm) and blue light (400–500 nm), which indicates that red light and blue light are the main energy sources for photosynthetic CO_2 assimilation (Pennisi et al., 2019). Therefore, the combination of red light and blue light is widely used as a light source for vegetable production in PFAL (Pennisi et al., 2019; Song et al., 2020) and there is a optimal R:B ratio for plant growth (Dou et al., 2017). However, the optimal R:B ratio is different for the growth of different vegetables. For instance, the yield of lettuce is maximized at RB3:1 (among RB1:2, RB1:1, RB2:1, RB3:1, RB4:1) (Pennisi et al., 2019). The leaf fresh weight of strawberry is significantly higher at RB3:2 than at other R:B ratios (with R:B ratio ranging 0.7-5.5) (Piovene et al., 2015). In addition to vegetable growth, the phytonutrient (essential oil, phenolic compounds and antioxidant compounds) in vegetables are also significantly affected by the change of R:B ratios (Dou et al., 2017).

Light intensity significantly affects the growth of vegetables, and vegetable yields no longer increases with the increased light intensity when the light intensity reaches a threshold. For example, the dry biomass of lettuce is significantly promoted by increasing the light intensity from 60-220 PPFD, regardless of the N concentration (Fu et al., 2017). Another study reported that when the light intensity increased to 350 PPFD the lettuce yield did not increase with the increased light intensity (Song et al., 2020). Moreover, the response of vegetable growth to light intensity is controlled by other cultivation conditions, such as NS. It is reported that the shoot dry weight was not affected by different light intensities (100-300 PPFD) at the EC of 1.0 and 2.0 dS m^{-1}, however, the dry weight increased with the increase of light intensity at the EC value of 3.0 dS m^{-1} (Lu et al., 2017). The secondary metabolite accumulation is also significantly affected by light intensity (Thoma et al., 2020). Moreover, the light intensity and light quality of light emitting diodes (LEDs) can be controlled. Therefore, it is possible to find the best light conditions for vegetable production by using LEDs in PFAL.

1.6. Research objectives

Yield and quality determine the economic value of vegetables. Antioxidants are widely found in vegetables as secondary metabolites that are beneficial to the human body. Therefore, increasing the antioxidant content of vegetables is conducive to improving the quality of vegetables. Basil is an important herb cultivated in PFAL, and basil is rich in antioxidants. Moreover, antioxidants are significantly affected by light conditions and NS. Besides, the discard of NS will cause the waste of resources and environmental pollution, therefore it is of great significance to improve the fertilizer use efficiency by optimizing NS management method and light conditions in PFAL. The objectives of this study are as follows:

1) To balance basil yield and antioxidant accumulation by adjusting the EC value of NS.
2) To improve basil yield and fertilizer use efficiency by determining an optimal combination of R:B ratio and ANQ.
3) To increase the antioxidant accumulation and reduce the nitrate content in basil by determining an optimal combination of R:B ratio and ANQ.

CHAPTER 2. Optimization of the yield, total phenolic content and antioxidant capacity of basil by controlling the electrical conductivity of the nutrient solution

2.1. Introduction

PFALs are considered a high-end agricultural model that has many advantages over traditional agriculture. These include scheduled production, shorter production cycle, local production, high vegetable quality, etc. In PFALs, environmental conditions such as the nutrient solution (NS), light intensity, photoperiod, and temperature are controlled by the operator, who can thus enhance the yield and content of antioxidants in plants through environmental control. In countries such as Japan, China, America, etc., PFALs have been being used to produce leafy greens, herbs, medicinal plants, and other vegetables (Kozai, 2018).

PFALs mainly use hydroponic cultivation with a NS that supplies the plants with fertilizers. Therefore, the composition of the NS is one of the most important environmental factors affecting plant growth and development. The total amount of available ions in the NS can be measured via EC monitoring, which is a relatively simple task (Trejo-Téllez and Gómez-Merino, 2012). Therefore, EC management is a common and important method widely used in hydroponic cultivation to effectively improve the yield and quality of vegetables (Lu et al., 2017; Wortman, 2015; Hosseini et al., 2021; Walters et al., 2018; Xu et al., 2009).

In practice, the EC is usually controlled at a target value in a recycling NS manually or automatically by EC control systems (Miller et al., 2020; Yolanda et al., 2018; Chen et al., 2022; Ibrahim et al., 2015). The EC value reflects the concentration of nutrients dissolved in the NS and is changed with the ratio of the volume of nutrients to the volume of water. During plant growth period, the EC level in the NS is influenced by plants because they absorb both nutrients and water from the NS (Trejo-Téllez and Gómez-Merino, 2012). The rise and fall of EC level, especially when it exceeds the tolerance of the plants, can cause nutrient stresses (excess or deficiency) that inhibit plant growth and development (Yolanda et al., 2018; Pandey et al., 2021). Controlling the EC of NS within an appropriate range is critical for plant production in PFALs. However, different crops have their specific ideal EC values (Sonneveld et al., 2009), thus the optimal EC level for a certain crop grown in PFALs needs to be determined prior to its commercial production.

Sweet basil is an important labiate plant used to produce essential oil (Wogiatzi et al., 2011; Nurzyńska-Wierdak et al., 2012), and it is also widely consumed as a culinary herb and medicinal plant (Simon et al., 1999), and the demand for basil is increasing worldwide. Basil is rich in secondary metabolites with antioxidant effects that can reduce the risk of many diseases, such as cardiovascular disease and cancer (Filip et al., 2016). Plant-derived antioxidants work by scavenging harmful free radicals derived from unhealthy habits, pollution, smoking, drugs, chemicals, etc., thus reducing the harm of oxidative stress against the human body (Sen and Chakraborty, 2011). Therefore, increasing the content of antioxidants such as phenolic compounds in basil by controlling environmental conditions during cultivation will improve the nutritional value of basil for humans. More and more growers are starting to produce basil in a PFAL because its high environmental controllability and sustainability allows for a reliable and stable supply of basil plants (Dou et al., 2018; Dou et al., 2019a; Dou et al., 2019b).

There are at least 65 species of basil (Makri and Kintzios, 2008) with numerous botanical varieties and the content of antioxidants and total phenolics varied widely among varieties (Moghaddam and Mehdizadeh, 2015; Javanmardi et al., 2003; Kwee and Niemeyer, 2011). In addition, changes in cultivation conditions, such as potassium supply (Nguyen et al., 2010), daily light integral (Dou et al., 2018), UV-B radiation (Dou et al., 2019a), photosynthetic photon flux density (Dou et al., 2019a; Hikosaka et al., 2021), red and blue light ratios (Dou et al., 2019b; Hikosaka et al., 2021), temperature and water stress (Al-Huqail et al., 2020), and salt stresses combined with storage periods (Bekhradi et al., 2015), affect secondary metabolites accumulation in basil plants, and different basil cultivars also respond differently to the same environmental conditions (Dou et al., 2019a; Dou et al., 2019b; Nguyen et al., 2010). In sweet basil, the antioxidant activity of different cultivars was influenced oppositely under the same EC treatments (Maggio et al., 2006), which showed that the antioxidant activity decreased in cultivar of “Genovese” but increased in cultivar of “Napoletano”, with increase in the EC from 2.0 dS m^{-1} to 4.0 dS m^{-1}. Another study reported that the accumulations of secondary metabolites including total polyphenols were significantly affected by different ECs (1.0, 2.0, 3.0 dS m^{-1}), and a significant interaction between the cultivars of sweet basil and the ECs was noted for phenolic acids (Ciriello et al., 2020).

The concentration of antioxidants and yield in biomass of plants may show opposite trends under the same environmental conditions, especially under stress conditions (Lu et al., 2017; Al-Huqail et al., 2020). It is reported that an EC value of 2.0 dS m^{-1} produces the maximum yield of wrinkled giant hyssop (*Agastache rugosa*), while producing relatively low levels of antioxidants; however, at an EC value of 0.5 dS m^{-1}, the content of antioxidants reaches the maximum, but at a relatively low yield (Lam et al., 2020). Therefore, the optimum EC value of NS often differs according to the purpose, e.g., obtaining either a high yield or high antioxidant content. Although it is sometimes difficult to maximize both yield and antioxidant content under the same environmental conditions, there are compromise methods that can balance these two aims. For instance, short-term root zone environmental control has been used to effectively balance vegetable yield and quality. Adjusting the NO_3^- content in the NS of lettuce decreased the NO_3^- content without decreasing the leaf fresh weight before harvest (Khan et al., 2018). In coriander, regulating the root zone temperature before harvest dramatically enhanced the content of antioxidants with only a small decline in yield (Nguyen et al., 2020).

The effects of EC value of NS on the growth and development of basil (Walters et al., 2018; Carrasco et al., 2007; Morano et al., 2017); on the antioxidant activity in basil (Maggio et al., 2006), perilla (Nguyen et al., 2021), and wild rocket (Bonasia et al., 2017); and on the phenolic compounds in *Agastache rugosa* (Lam et al., 2020) and tomato (Moya et al., 2017) have been reported. However, there are no studies on the use of short-term regulation of the EC value of NS before harvest to balance yield and accumulation of antioxidants in vegetables and herbs. Moreover, short-term root zone environmental control is an effective method to balance vegetable growth and quality (Khan et al., 2018; Nguyen et al., 2020). We hypothesized that short-term EC treatment before harvest can balance the growth and secondary metabolite accumulation of basil. Therefore, the present study aimed firstly to determine the respective optimal EC values of NS for maximizing yield and accumulation of antioxidants, and then to develop a short-term EC management method that can be applied prior to harvest to balance these two aspects of basil plants grown in PFALs.

2.2. Materials and Methods

2.2.1. Plant material

Sweet basil (*Ocimum basilicum* L. var. *basilicum* L. cv. Genovese, Takii & Co., Ltd., Japan) seeds were sown in sponge cubes (2.3 × 2.3 × 2.8 cm, 14.8 cm^3) in a cultivation room of a PFAL. The germinated seeds were placed under a photosynthetic photon flux density (PPFD) of 200 µmol m^{-2} s^{-1} with a photoperiod of 16 h per day using LED lamps (Plant growth light, 18W, SananBio, Xiamen, China). The seedlings were irrigated with an NS consisting of the following nutrients: N 21%, P_2O_5 8%, K_2O 27%, MgO 4%, CaO 23%, Fe 0.18%, Cu 0.002%, Zn 0.006%, Mo 0.002%, MnO 0.1%, and B_2O_3 0.1% (Otsuka hydroponic composition, OAT Agrio Co., Ltd., Tokyo, Japan) (Sun et al., 2016). The EC and pH of the NS were measured by a multi-parameter meter (Eutech PCTestr 35 multi-parameter pocket tester; Eutech Instruments Pte Ltd., Singapore) and adjusted to 2.0 dS m^{-1} and 6.5, respectively, during the nursery stage.

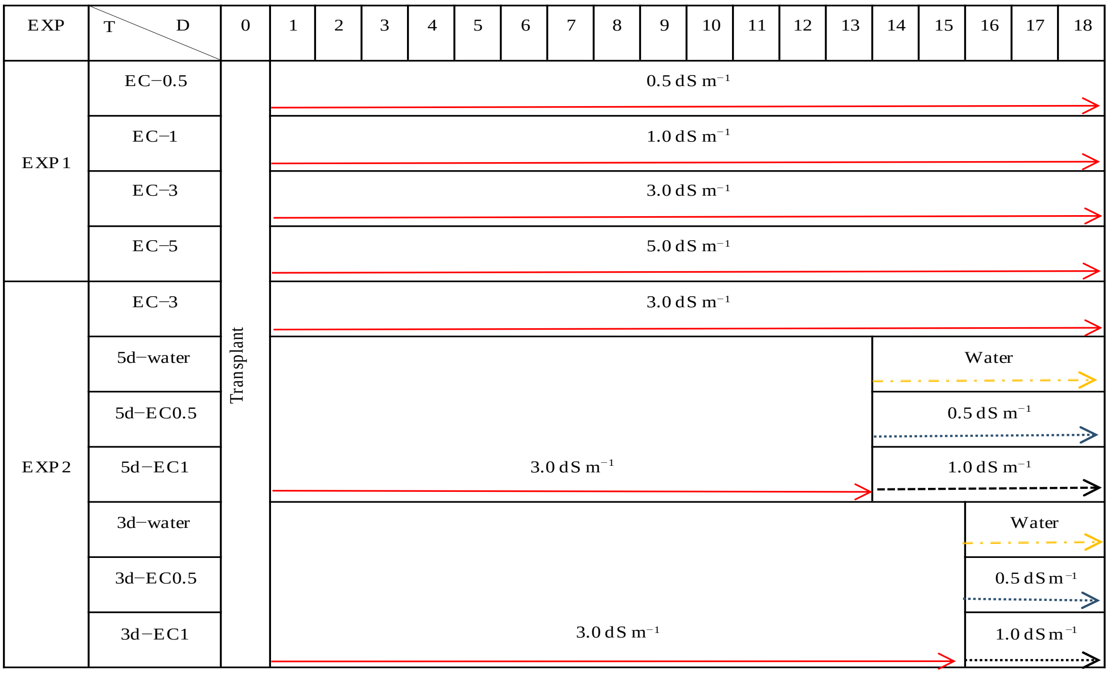
2.2.2. Treatments

Two experiments were conducted starting at 16 days after germination (Figure 1). In the first experiment (Experiment 1), sweet basil seedlings were transplanted into four plastic cultivation containers (70 × 46 × 11 cm, LWH, 35.4 L) containing NS with EC values of 0.5, 1.0, 3.0, and 5.0 dS m^{-1}. The NS composition was the same as mentioned above, and the nutrient concentrations of different ECs are shown in Table 1. The ratio of the concentration of NO_3^-/NH_4^+ was 10:1.

After Experiment 1, the second experiment (Experiment 2) was conducted, by transplanting seedlings into seven plastic cultivation containers (same containers as above) containing NS with an EC value of 3.0 dS m^{-1} and incubating them for 13 or 15 days. Then the plants were treated with water or NS with EC values of 0.5 and 1.0 dS m^{-1} for another 5 or 3 days, before harvest. The control consisted of seedlings in NS with an EC value of 3.0 dS m^{-1} and incubated for 18 days after transplanting (Figure 1). Experiment 2 was carried out after the results of Experiment 1 were obtained. Air pumps were used to supplement oxygen in both experiments.

Thirty-two seedlings were grown under each treatment in both experiments, and each container contained 30 L NS. The plant density was 99 plants m^{-2}. To facilitate the replenishment of NS, a 100× concentrated NS was made, and the proportions of all elements in the concentrated NS were the same. The NS was adjusted every two days to maintain the target EC value and NS volume (30 L). At the same time, the pH of the NS was adjusted to 6.5. Light was provided by the same LED lamps as described above, with a PPFD of 200 ± 15 μmol m^{-2} s^{-1}. The PPFD was measured at the surface of the cultivation panel using a light meter (LI 250A, LI-190R; Li-Cor Inc., Lincoln, NE, USA). The light spectrum is shown in Supplementary Figure S1. Temperature, photoperiod, CO_2 concentration, and relative humidity were set to 21 °C/24 °C (dark/light), 8/16 h (dark/light), 1500 ppm, and 60%–80%, respectively. The basil plants were harvested at 34 days after sowing. Both experiments were repeated twice.

Figure 2.1. Schematic diagram of the treatments imposed on basil plants cultivated for 18 days in experiment 1 and experiment 2.



Treatment code (T): EC−0.5, EC−1, EC−3, and EC−5 represent nutrient solution with electrical conductivities (ECs) of 0.5, 1.0, 3.0, and 5.0 dS m^{-1}, respectively. 5d−water, 5d−EC0.5, and 5d−EC1 represent treatments with water, EC−0.5, and EC−1 imposed 5 days before harvest, respectively. 3d−water, 3d−EC0.5, and 3d−EC1 represent treatments with water, EC−0.5, and EC−1 imposed 3 days before harvest, respectively. D: days of cultivation. EXP: experiment.

Table 2.1. Nutrient concentration of different EC treatments.

Nutrients	Unit	EC (dS m^{-1})			
		0.5	1.0	3.0	5.0
N	mM	3.70	7.40	22.20	37.00
P		0.34	0.68	2.04	3.40
K		1.52	3.04	9.12	15.20
Ca		0.82	1.64	4.92	8.20
Mg		0.37	0.74	2.22	3.70
Fe	µM	10.18	20.36	61.07	101.79
Mn		2.80	5.60	16.80	28.00
Zn		0.12	0.24	0.73	1.22
Cu		0.06	0.13	0.38	0.63
Mo		0.04	0.08	0.25	0.42
B		5.93	11.85	35.56	59.26

2.2.3. Measurements

2.2.3.1. Growth parameters

The basil plants were harvested at 18 days after treatment. Leaf, stem, and root fresh weights were measured immediately after harvesting. To measure dry weights, the leaf, stem, and root samples were oven-dried at 80 °C for 3 days to a constant weight before measurements. Total leaf area was measured with a leaf area meter (Li–3000, Li-Cor, Lincoln, NE, USA). Leaf mass per area (LMA) was determined as leaf dry weight divided by leaf area. Each parameter consisted of measurements from 12 samples from each treatment.

2.2.3.2. Gas-Exchange parameters

The net photosynthetic rates (P_n), stomatal conductance (Gs), and transpiration rate (Tr) of basil leaves were determined using a gas-exchange system (LI-6400-40, Li-Cor, Inc., Lincoln, NE, USA) at 34 days after sowing. Water use efficiency of photosynthesis (WUE_{Pn}) was calculated as $WUE_{Pn} = Gs \div P_n$ (Prehn et al., 2010). The youngest fully expanded leaf from each treatment was used for measurements (Xu et al., 2021). Eight samples were measured from each treatment. The light intensity, CO_2 concentration, relative humidity, and leaf temperature inside the leaf chamber were set to 200 PPFD, 1500 mmol mol^{-1}, 65%, and 23 °C, respectively.

2.2.3.3. Measurements of TPC and antioxidant capacity in basil leaves

Extraction. A frozen leaf sample (1 g each) was homogenized with 80% (*v*/*v*) methanol (5 mL) for 1 min. The sample was centrifuged (10,000× *g*, 4 °C) for 30 min. After centrifuging, the supernatant was transferred to a 10 mL graduated cylinder and made up to 6 mL with 80% methanol and then stored at −30 °C for further analysis.

TPC evaluation. The colorimetric analysis of Folin−Ciocalteu (Nguyen et al., 2020) was used to determine the TPC of basil leaves, using gallic acid as the calibration standard. An aliquot of 0.25 mL of test sample or gallic acid solution (0, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mg mL^{-1}) was added to 1.25 mL of 10% Folin−Ciocalteu reagent, followed by 1 mL of 7.5% sodium carbonate solution, and then this was mixed thoroughly at room temperature. After 1 h, the absorbance of mixed solution was measured at 765 nm with a spectrophotometer (ASV11D, As One, Corp., Osaka, Japan). The results are expressed as milligram gallic acid equivalents per gram fresh weight (mg GAE g^{-1} FW).

Total antioxidant capacity evaluation. Free radical scavenging activity was evaluated using a 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay (Gonçalves et al., 2013) with some modifications. An aliquot of 50 µL of test sample or Trolox solution (0, 200, 400, 600, 800, and 1000 µM) was added to 2 mL of a DPPH solution (80 µM) in methanol and mixed thoroughly at room temperature. After 30 min under dark condition, the absorbance of mixed solution was measured at 517 nm with a spectrophotometer (ASV11D, As One, Corp., Osaka, Japan), and the results are expressed as milligram Trolox equivalents per gram fresh weight (mg TE g^{-1} FW).

2.2.3.4. Measurements of photosynthesis-related pigments in basil leaves

The chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid concentrations of basil leaves were determined immediately after harvest, following the methods described previously (Nguyen et al., 2020). The weighted samples were placed into a glass vial containing 2 mL *N*,*N*-Dimethylformamide (DMF) and immediately put in darkness at 4 °C for 36 h. A spectrophotometer (SH-1300Lab, Corona Electric Co., Ltd., Ibaraki, Japan) was used to measure the absorbance of the solution at different wavelengths (645, 663, 480 nm) using DMF as a blank. The contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were calculated by the formula described previously (Nguyen et al., 2020).

2.2.4. Statistical analysis

For each treatment, four to twelve replicates were obtained to evaluate different parameters. The data were subjected to analysis of variance and the means were compared between treatments using Tukey’s test in SPSS statistical software (IBM SPSS Statistics, Version 19.0. Armonk, NY, USA: IBM Corp.). A p-value < 0.05 was considered significant.

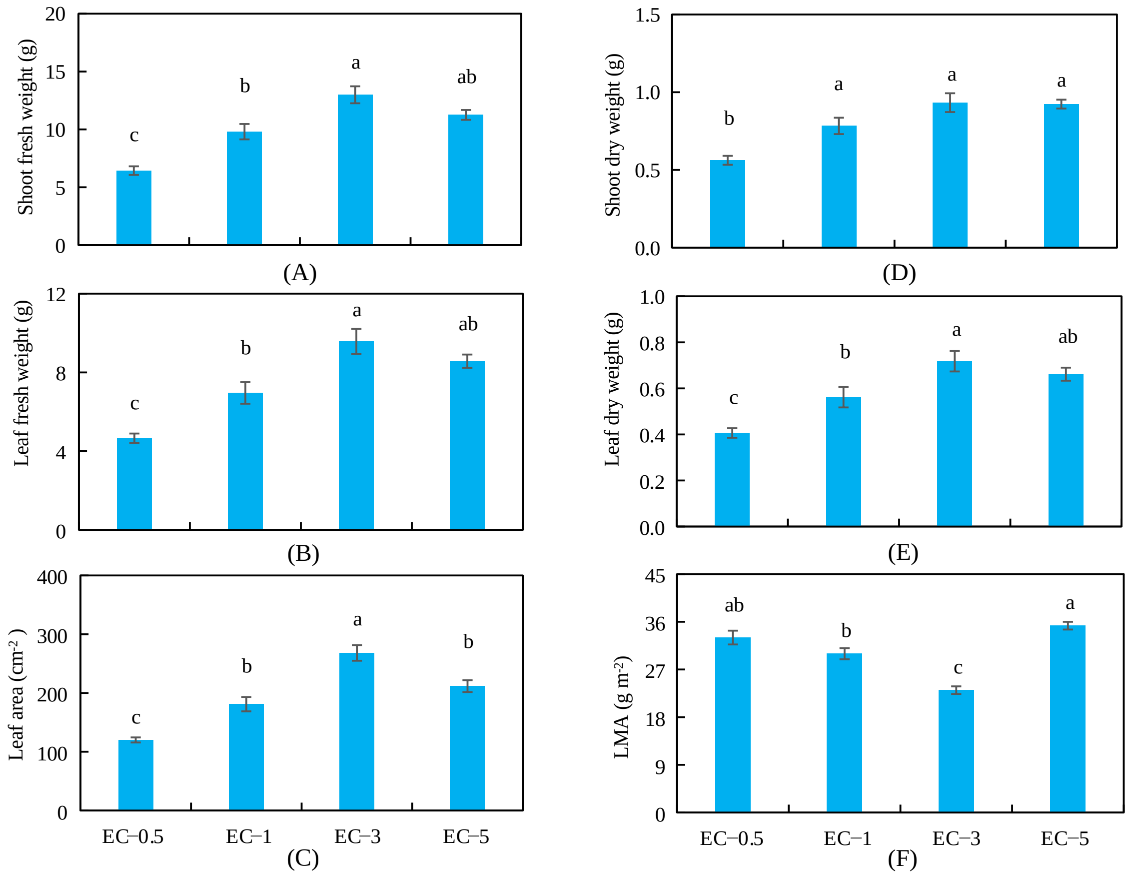
2.3. Results

2.3.1. Experiment 1

2.3.1.1. Plant growth under different EC treatments

The shoot and leaf FW and leaf area increased significantly when the EC value was increased from 0.5 to 3.0 dS m^{-1} and then decreased slightly when the EC value was further increased to 5.0 dS m^{-1} (Figure 2A–C). The shoot and leaf DW followed a similar trend as the shoot and leaf FW; however, the differences in shoot DW at EC values of 1.0, 3.0, and 5.0 dS m^{-1} were not significant (Figure 2D,E). The LMA decreased as EC increased from 0.5 to 3.0 dS m^{-1} and then increased significantly as EC increased from 3.0 to 5.0 dS m^{-1} (Figure 2F). The morphological characteristics of basil plants in each treatment before harvest are shown in Supplementary Figure S2.

Figure 2.2. Shoot fresh weight (A), leaf fresh weight (B), leaf area (C), shoot dry weight (D), leaf dry weight (E), and leaf dry mass per area (LMA) (F) of basil plants under 4 different EC treatments at 34 days after sowing.



The error bars represent SEs (n = 12). Different lowercase letters represent significant differences among different EC treatments based on Tukey's test at $p < 0.05$.

The effects of different EC treatments on other plant growth parameters and the relationship between the plant DW and the leaf area of basil are shown in Supplementary Table S1 and Supplementary Figure S3, respectively. The FW of stems and roots and plant height of basil increased significantly as EC was increased from 0.5 to 3.0 dS m^{-1} and any further increase in EC produced no further changes in these parameters (Table S1). The statistical analysis showed a positive and highly significant relationship between plant DW and leaf area (Figure S3B).

2.3.1.2. Net photosynthetic rate and chlorophyll and carotenoid concentrations of basil leaves under different EC treatments

The net photosynthetic rates, chlorophyll a, chlorophyll b, carotenoid contents, the values of chlorophyll a/chlorophyll b and total chlorophyll/carotenoid of basil leaves did not differ significantly among the different EC treatments (Table 2). The stomatal conductance (Gs), transpiration rate (Tr), and water use efficiency of photosynthesis (WUE_{Pn}) are shown in Supplementary Table S2, and there were also no significant differences in these parameters under different EC treatments.

Table 2.2. The net photosynthetic rate, chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid contents, the values of Chl a/Chl b, and total Chl/carotenoid of basil leaves under 4 different EC treatments at 34 days after sowing in experiment 1.

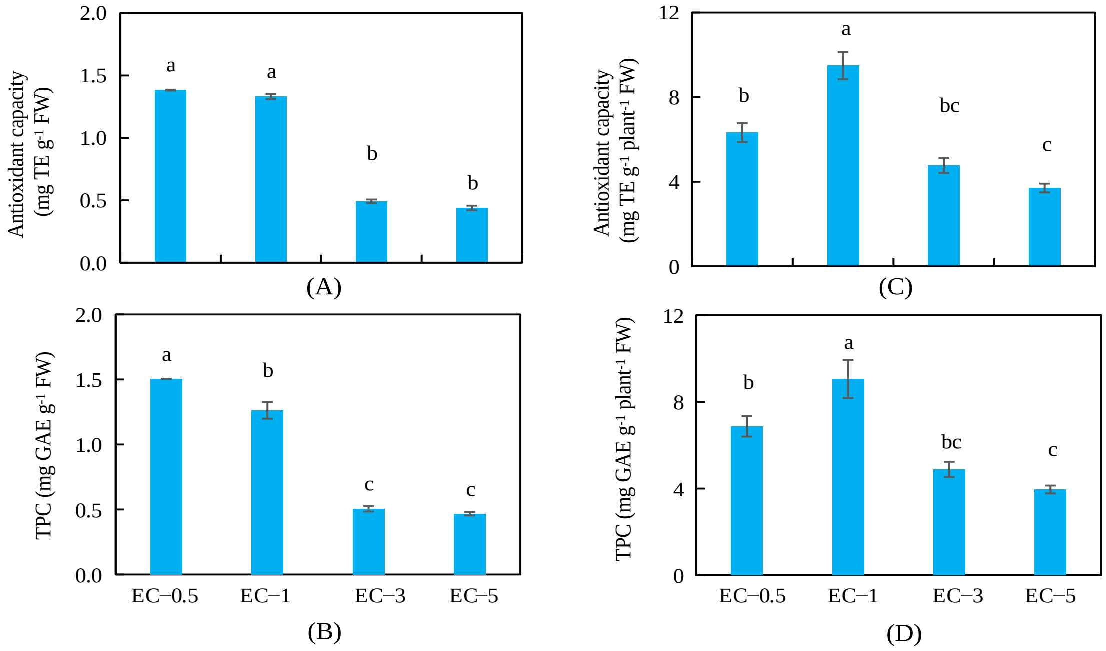
Treatment Code	Net Photosynthetic Rate (μmol CO_2 m^{-2} s^{-1})	Chl a (mg g^{-1} FW^{-1})	Chl b (mg g^{-1} FW^{-1})	Carotenoid (mg g^{-1} FW^{-1})	Chl a/Chl b	Total Chl/Carotenoid
EC−0.5	7.80 ± 0.61 [y]	0.49 ± 0.03	0.18 ± 0.01	0.11 ± 0.01	2.76 ± 0.04	6.24 ± 0.03
EC−1	8.36 ± 0.26	0.46 ± 0.03	0.16 ± 0.01	0.10 ± 0.01	2.81 ± 0.04	6.18 ± 0.11
EC−3	7.56 ± 0.09	0.53 ± 0.04	0.18 ± 0.01	0.11 ± 0.01	2.86 ± 0.09	6.18 ± 0.05
EC−5	8.25 ± 0.24	0.43 ± 0.01	0.16 ± 0.00	0.10 ± 0.00	2.68 ± 0.04	6.00 ± 0.10
ANOVA [z]	ns	ns	ns	ns	ns	ns

[z] Results of analysis of variance (ANOVA) are shown. NS, non-significant. [y] Each value is the mean ± SE of four to six replicates. Chl a and Chl b represent chlorophyll a and chlorophyll b, respectively.

2.3.1.3. Antioxidant capacity and TPC of basil leaves under different EC treatments

The antioxidant capacities of basil leaves were significantly higher at ECs of 0.5 and 1.0 dS m^{-1} compared with those at ECs of 3.0 and 5.0 dS m^{-1}, and no significant differences were observed in this parameter between ECs of 0.5 and 1.0 dS m^{-1} or between ECs of 3.0 and 5.0 dS m^{-1} (Figure 3A). The TPC decreased as the EC was increased from 0.5 to 3.0 dS m^{-1}; however, there was no further decrease in the TPC when the EC was increased from 3.0 to 5.0 dS m^{-1} (Figure 3B). The antioxidant capacity and the TPC in the leaves of the whole plant both increased significantly as EC was increased from 0.5 to 1.0 dS m^{-1} (mainly due to the difference in leaf FWs, Figure 1B) and then decreased with the increase in EC value at 3.0 and 5.0 dS m^{-1} (Figure 3C,D).

Figure 2.3. The antioxidant capacity in basil leaves (A), total phenolic content (TPC) (B) in basil leaves, antioxidant capacity in leaves of the whole plant (C), and TPC in leaves of the whole plant (D) under 4 different EC treatments at 34 days after sowing.



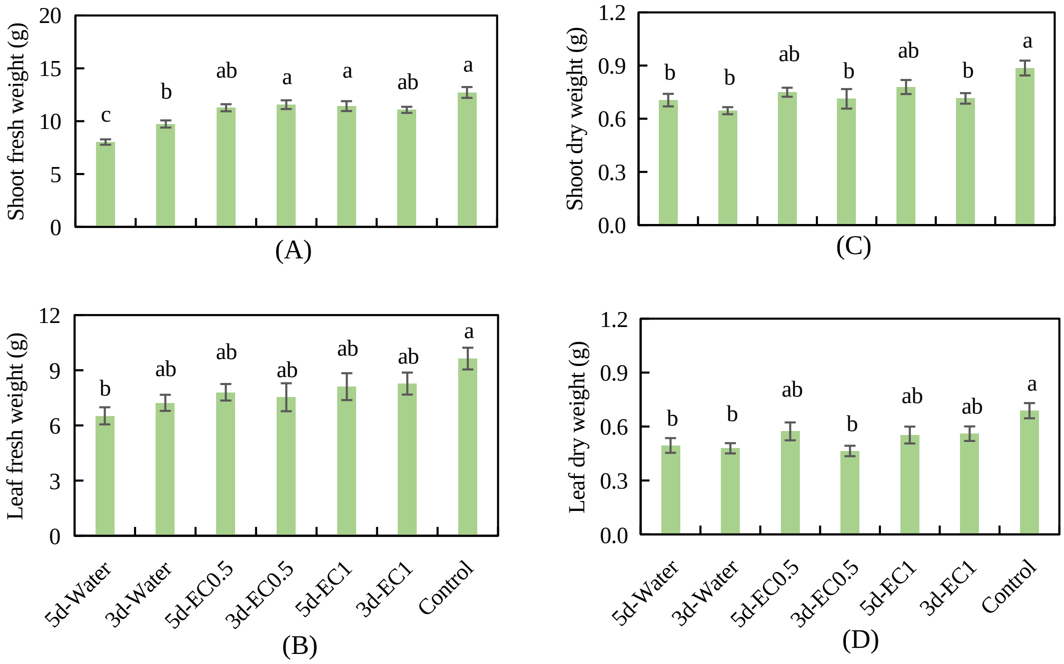
The error bars represent SEs (n = 8). Different lowercase letters represent significant differences among different EC treatments based on Tukey's test at $p < 0.05$.

2.3.2. Experiment 2

2.3.2.1. Plant growth under different short-term EC treatments

No significant decrease was observed in shoot FW in response to treatments of EC0.5 and EC1 for 3 or 5 days; however, shoot FW did decrease significantly in response to water treatment for 3 and 5 days, compared with the control (Figure 4A). Leaf FW did not decrease significantly in response to all treatments except that of water for 5 days (Figure 4B), compared with the control. Shoot DW did not decrease significantly in response to EC0.5 and EC1 treatments for 5 days; however, shoot DW decreased significantly in response to the other treatments, compared with the control. Leaf DW did not decrease significantly in response to the EC0.5 treatment for 5 days or the EC1 treatment for 3 and 5 days; however, leaf DW decreased significantly in response to the other treatments (Figure 4C,D), compared with the control. Shoot and leaf DWs did not change significantly in response to treatment duration under any of the EC values.

Figure 2.4. Shoot fresh weight (A), leaf fresh weight (B), shoot dry weight (C), and leaf dry weight (D) of basil plants under different short-term EC treatments at 34 days after sowing.



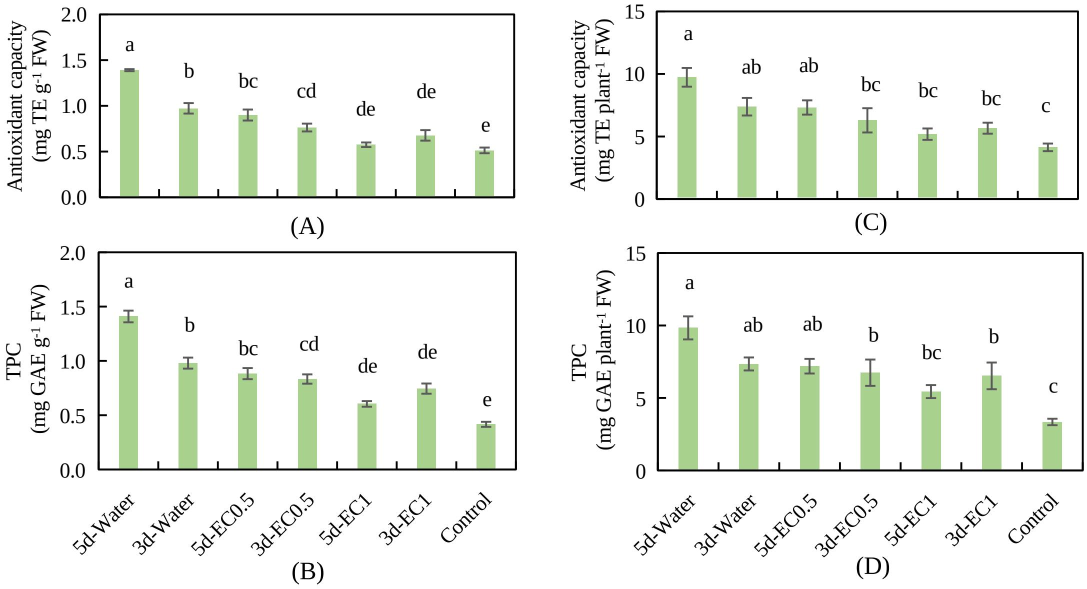
The error bars represent SEs (n = 12). Different lowercase letters represent significant differences among different EC treatments based on Tukey's test at $p < 0.05$.

2.3.2.2. Antioxidant capacity and TPC under different short-term EC treatments

Compared with the control, both antioxidant capacity and TPC were significantly improved by the water and EC0.5 treatments for 3 or 5 days but were not influenced by the EC1 treatment for either 3 or 5 days (Figure 5A,B). The highest antioxidant capacity and TPC were achieved with the 5d-water treatment, followed by 3d-water, 5d-EC0.5, 3d-EC0.5, and 3d-, 5d-EC1 treatments (Figure 5A,B). The effect of different treatment durations on antioxidant capacity and TPC varied among the different ECs. In the water treatment, longer treatment duration (5 days) enhanced the antioxidant capacity and TPC significantly more than in the shorter treatment duration (3 days). However, these two parameters were not affected significantly by the treatment duration at ECs of 0.5 and 1.0 dS m^{-1} (Figure 5A,B).

The trends observed for antioxidant capacity and TPC in the leaves of the whole plant as affected by short-term EC treatments were similar (Figure 5C,D). The highest antioxidant capacity and TPC in the leaves of the whole plant were achieved in the 5d-water, 3d-water, and 5d-EC0.5 treatments, and these levels were significantly higher than those of the control (Figure 5C,D).

Figure 2.5. The antioxidant capacity in basil leaves (A), total phenolic content (TPC) in basil leaves (B), antioxidant capacity in leaves of the whole plant (C), and TPC in leaves of the whole plant (D) under different EC treatments at 34 days after sowing.



The error bars represent SEs ($n = 8$). Different lowercase letters represent significant differences among different EC treatments based on Tukey's test at $p < 0.05$.

The effects of different EC treatments on the leaf area and the growth of stems and roots of basil plants are shown in Supplementary Table S3.

Compared with the control, the leaf area did not decrease significantly in response to 3d-EC0.5 and 5d-EC1 treatments (Table S3); the stem FW did not decrease significantly in response to the 5d-EC0.5 and 5d-EC1 treatments; and the stem DW was not decreased significantly in response to the 5d-EC1 treatment (Table S3). The leaf area and stem FW and DW were not significantly affected by the different durations of the EC treatments of water, 0.5, and 1.0 dS m^{-1}. The root FW did not differ significantly among all treatments; however, the root DW did increase significantly in response to the 5d-water and 5d-EC0.5 treatments as compared with the control (Table S3).

Compared with the control, the leaf FW had the highest reduction in percentage under the treatment of 5d-water, followed by 3d-water, 3d-EC0.5, 5d-EC0.5, 5d-EC1, and 3d-EC1. Moreover, the highest percentage increase of the antioxidant capacity (per unit g FW), TPC (per unit g FW), antioxidant capacity in the leaves (per plant g FW), and TPC in the leaves (per plant g FW) were achieved under the treatment of 5d-water, followed by 3d-water, 5d-EC0.5, 3d-EC0.5, and 3d-, 5d-EC1, compared with the control (Table S4).

2.4. Discussion

2.4.1. Growth and yield of basil under different EC treatments

Our results show the highest shoot FW, leaf FW, shoot DW, and leaf DW of basil at an EC of 3.0 dS m^{-1}; and the shoot FW, leaf FW, and leaf DW of basil at ECs of 0.5 and 1.0 dS m^{-1} were significantly lower than those at an EC of 3.0 dS m^{-1} (Figure 1A,B,D,E). Similarly, in perilla, the shoot DW, leaf DW, and leaf area were significantly lower at an EC of 1.0 dS m^{-1} compared with those at an EC of 3.0 dS m^{-1} (Lu et al., 2017). It is reported that the leaf fresh and dry weights of basil plant decreased significantly when the EC value was decreased from 1.2 to 0.5 dS m^{-1} (Hosseini et al., 2021). These results indicate that EC levels lower than 1.0 dS m^{-1} may have an adverse effect on basil and perilla plants (Lu et al., 2017; Hosseini et al., 2021). However, the optimal EC levels for maximizing growth and yield of these plants depend on cultivars and other environmental conditions. For example, an EC of 2.0 dS m^{-1} was optimal for cultivar of "Aroma 2", but an EC of 3.0 dS m^{-1} was optimal for cultivar of "Italiano Classico" in basil plants (Ciriello et al., 2020). The yields of green perilla were similar under EC 2.0 and 3.0 dS m^{-1} when the PPFD was 100 μmol m^{-2} s^{-1}, but its yield was significantly higher at an EC of 3.0 dS m^{-1} than at an EC of 2.0 dS m^{-1} when the PPFD was increased to 200 or 300 μmol m^{-2} s^{-1} (Lu et al., 2017).

Different vegetables also respond differently to changes in EC values. For instance, the growth and yield of pakchoi are reduced more by low ECs (0, 0.3, 0.6, and 1.2 dS m^{-1}) than by higher ECs (1.8 and 2.4 dS m^{-1}) (Ding et al., 2018). Similarly, in *Crepidiastrum denticulatum* (the common name: e-go-deulppae-gi in Korean), the growth and yield are reduced more by low ECs (0.5, 1.0, and 1.5 dS m^{-1}) than by higher ECs (2.0 and 2.5 dS m^{-1}) (Park et al., 2016), while the shoot fresh and dry weights of lettuce decrease significantly in response to increasing the EC of the NS from 1.4 to 3.0 dS m^{-1} (Samarakoon et al., 2006). Moreover, in our study, ECs of 3.0

and 5.0 dS m^{-1} did not result in significant differences in shoot FW, leaf FW, shoot DW, and leaf DW (Figure 1A,B,D,E); however, the shoot FW and shoot DW of pakchoi at an EC of 4.8 dS m^{-1} were significantly higher than those at an EC of 2.4 dS m^{-1} (Ding et al., 2018). Even within basil plants, the effects of EC on plant growth and yield could be different. One study has reported that basil plants had the best yield and growth indexes under the EC value of 2.8 dS m^{-1} (among EC of 2.2, 2.5, 2.8, 3.1 dS m^{-1}) (Morano et al., 2017); however, the growth of basil plants was not affected by the EC values (among EC of 0.5, 1.0, 2.0, 3.0, 4.0 dS m^{-1}) (Walters et al., 2018); the fresh yield of basil plants under an EC of 1.0 dS m^{-1} was significantly higher than that under an EC of 3.0 dS m^{-1} (cultivar “Eleonora”) (Ciriello et al., 2020). The different results may be caused by the difference in growth conditions—for instance, different PPFD values: 378 µmol m^{-2} s^{-1} (Walters et al., 2018) vs. 200 µmol m^{-2} s^{-1} in our study and in the Dou et al. report (Dou et al., 2018); different plant densities: 317 plants m^{-2} (Ciriello et al., 2020) vs. 99 plants m^{-2} in our study, which referred to the recommended plant density of 100 plants m^{-2} in hydroponic basil cultivation (Abbas, 2014).

In our study, different ECs produced differences in basil biomass; however, the net photosynthetic rate and the photosynthesis-related pigment levels were not affected by the EC treatments (Table 2). Similarly, in perilla and *Crepidiastrum denticulatum*, the net photosynthetic rates are unaffected by differences in EC conditions (Lu et al., 2017; Park et al., 2016). In contrast, the net photosynthetic rate and relative chlorophyll content of pakchoi are significantly affected by different EC conditions (Ding et al., 2018). Moreover, plant DW of basil was highly positively related to leaf area (Figure S3). Therefore, at ECs that affected the leaf area of basil (0.5, 1.0, and 3.0 dS m^{-1}), the photo assimilates of the whole plant were influenced. This phenomenon was also observed in tomato plants under different EC conditions (1.3 to 8.8 dS m^{-1}) (Schwarz et al., 2002).

2.4.2. TPC and antioxidant capacity of basil leaves under different EC treatments

Nutrient stress, either insufficient or excessive amounts of essential nutrients that inhibit plant growth and development (Zeng et al., 2014), could enhance accumulation of antioxidants. It is reported that the accumulation of antioxidants in tomato and pepper is significantly higher at ECs of 4.5 and 4.4 dS m^{-1} compared with that at the lower EC of 3.5 dS m^{-1} (Moya et al., 2017; Amalfitano et al., 2017). However, in our study, the antioxidant capacity and TPC of basil were significantly higher under low ECs of 0.5 and 1.0 dS m^{-1} compared with those under high ECs of 3.0 and 5.0 dS m^{-1} (Figure 3A,B). Specifically, the antioxidant capacity and TPC at EC of 0.5 dS m^{-1} were increased by 181.6% and 200.0% over those at EC of 3.0 dS m^{-1}, respectively, and similarly, the two parameters at EC of 1.0 dS m^{-1} were increased by 171.4% and 152.0% over those at EC of 3.0 dS m^{-1}, respectively. Nutrient deficiency stress can promote the accumulation of phenolic compounds, as shown by the relatively high content of rosmarinic acid (a phenolic compound) in basil plants grown in a NS with low N content (Kiferle et al., 2013). The phenolic compound in rice increases significantly in a NS with low levels of N, P, and K (Chishaki et al., 1997). Nutrient limitation also significantly promoted the content of phenolic compounds and the antioxidant capacity in basil (Jakovljević et al., 2019). Similarly, the phenolic compounds and antioxidants in lettuce increase significantly in a NS with low levels of N and P (Galieni et al., 2015). On the other hand, it seems an EC of 5.0 dS m^{-1} did not cause nutrient excess stress in the present study, as evidenced by the similar antioxidant capacities and TPCs of basil plants grown at ECs of 3.0 and 5.0 dS m^{-1} (Figure 3A,B). Similarly, the antioxidant enzyme activity of pakchoi plants did not increase (Ding et al., 2018) when the EC was increased from 1.2 or 2.4 dS m^{-1} to 4.8 dS m^{-1} (≈5.0 dS m^{-1}); however, the antioxidant enzyme activity of pakchoi plants did increase significantly when the EC was further increased to 9.6 dS m^{-1}.

The effects of EC on the accumulation of secondary metabolites vary with different cultivars of sweet basil. For example, the total phenolic acids were not affected by different salt stresses (ECs of 1.23, 4.87 and 8.62 dS m^{-1}) in green (var. Green Iranian) and purple (var. Purple Iranian) basils (Bekhradi et al., 2015). However, the TPC was significantly enhanced by EC of 3.0 dS m^{-1}, compared with that at EC of 1.0 dS m^{-1} in cultivar of "Aroma 2", but showed no changes with different ECs in cultivar of "Eleonora" (Ciriello et al., 2020). The antioxidant activity at high EC (4.0 dS m^{-1}) was higher than that at low EC (2.0 dS m^{-1}) in sweet basil cultivar of "Napoletano" (Maggio et al., 2006). Different from the results mentioned above, the TPC and antioxidant capacity were significantly improved by low ECs (0.5 and 1.0 dS m^{-1}) compared with high ECs (3.0 and 5.0 dS m^{-1}) in the sweet basil cultivar used in the present study.

From the two aspects of the yield and the accumulation of antioxidants in basil leaves, it is found that the leaf FW of basil at ECs of 0.5 and 1.0 dS m^{-1} was decreased by 51.3% and 27.3%, compared with that under EC of 3.0 dS m^{-1}, whereas the antioxidant capacity and TPC in the leaves of the whole plant were increased by 32.5% and 40.8% at EC of 0.5 dS m^{-1} and by 99.0% and 85.7% at EC of 1.0 dS m^{-1}, compared with those at EC of 3.0 dS m^{-1}. These differences were because the antioxidant capacity and TPC at ECs of 0.5 and 1.0 dS m^{-1} are largely higher than those under ECs of 3.0 dS m^{-1} (Figure 3C,D). Achieving a balance between yield and the accumulation of antioxidants in vegetables is important but difficult in practice. It has been shown that the yield and quality of vegetables can be controlled using methods of short-term regulation of root zone environment (Khan et al., 2018; Nguyen et al., 2020); thus, Experiment 2 was conducted, and the results are discussed in the following section.

2.4.3. Balance of yield and the accumulation antioxidants of basil under short-term EC treatments

Due to nutrient deficiency, relative long-term low-EC treatment reduced plant biomass in pakchoi (Ding et al., 2018) and *Crepidiastrum denticulatum* (Park et al., 2016) and increased phenolic compounds in basil (Kiferle et al., 2013; Jakovljević et al., 2019) and rice (Chishaki et al., 1997). In experiment 2, short-term low-EC treatments were applied to find a cultivation method that may balance yield and antioxidant accumulation in basil. The results show that compared with other treatments, the 5d-EC0.5 and 3d-water treatments achieved relative better results: yields were not significantly lower than those of the control, while promoting the accumulation of antioxidants. Specifically, compared with the control, the 5d-EC0.5 treatment did not reduce leaf FW, while increasing the TPC in the leaves (per plant g FW) and antioxidant capacity in the leaves (per plant g FW) by 115.3% and 66.0%, respectively; and the 3d-water treatment increased the same parameters by 119.9% and 67.4%, respectively (Table S4), while maintain leaf FW (Figure 4B). Such degrees of increase in antioxidants production would be a great benefit for improving the market value of the products. In addition, the increase in antioxidant capacity may be attributed to the increase in TPC because total phenolics play a major role in the antioxidant capacity in plants (Gonçalves et al., 2013; Velioglu et al., 1998). The results indicate that short-term EC treatments in the root zone environment can be used to balance the yield and the accumulation of antioxidants, which is based on the principle that stress promotes the accumulation of antioxidants in plants (Akula and

Ravishankar, 2011; Isah, 2019; Naikoo et al., 2019). In our study, short-term EC treatments promoted the accumulation of antioxidants without decreasing the yield of basil, which provides a new and relatively simple means of growing basil for PFAL operators.

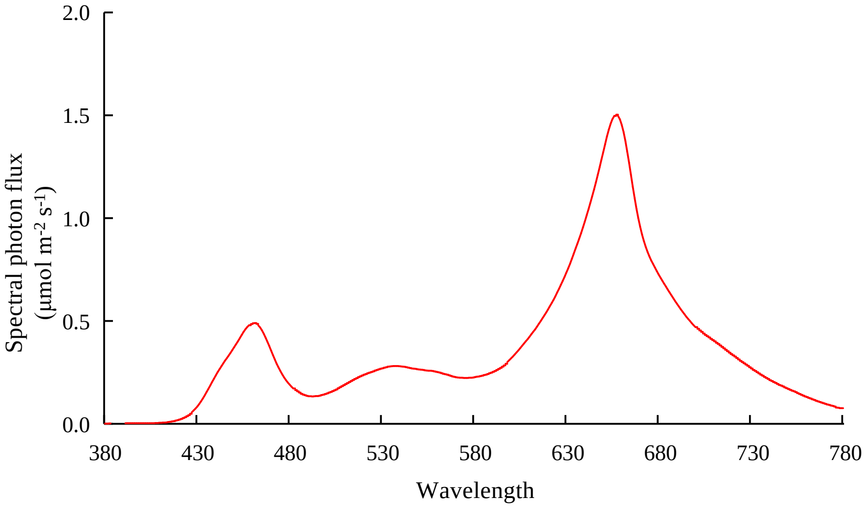
Table S4 shows that compared with the control, the 5d-water treatment accumulated much higher levels of antioxidants in basil, although the leaf FW was significantly reduced by 24.4%. Based on the results of the 5d-water and 3d-water treatments, a short-term treatment of 4d-water may achieve a higher accumulation of antioxidants compared with 3d-water, while experiencing a lower decline in yield than 5d-water. This hypothesis requires further research in the future.

2.5. Conclusions

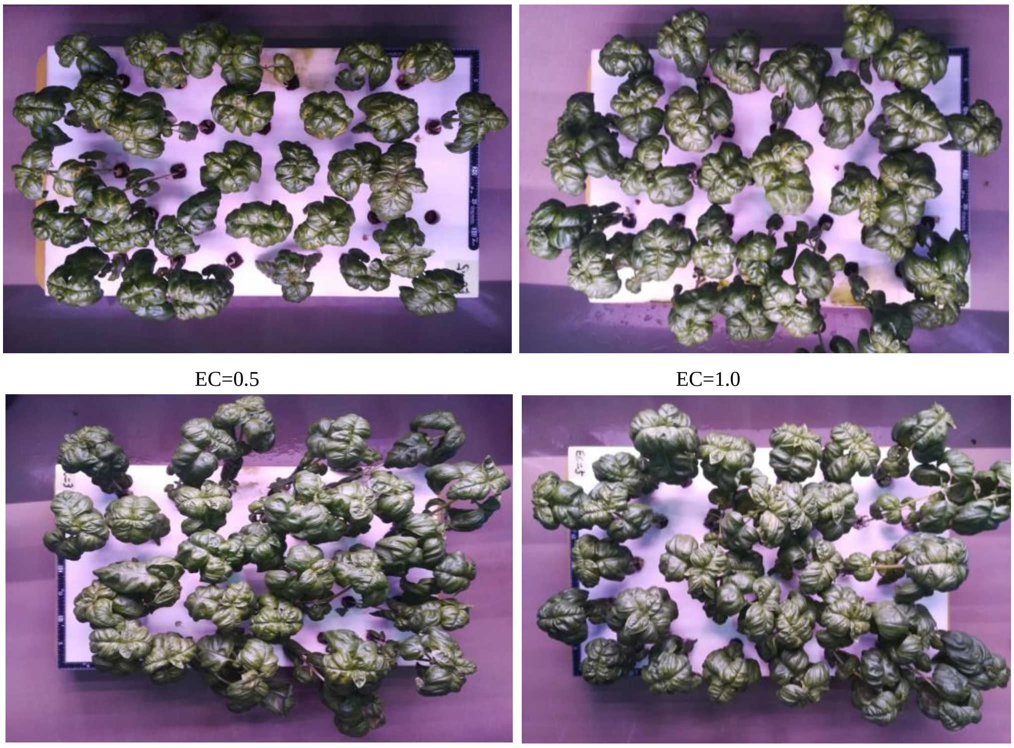
Different electrical conductivity treatments affected the growth, total phenolic content, and antioxidant capacity of basil grown in a plant factory with artificial lighting. An EC of 3.0 dS m^{-1} significantly promoted the yield of basil, but the level of accumulation of antioxidants was lower compared with those in other EC treatments. ECs of 0.5 and 1.0 dS m^{-1} significantly increased the antioxidant capacity and TPC of basil, while significantly inhibiting yield. Short-term EC treatments before harvest were imposed to balance the yield and antioxidant accumulation of basil. The treatments with EC of 0.5 dS m^{-1} for 5 days or water for 3 days before harvest can promote the antioxidant capacity and TPC in basil leaves without sacrificing yield significantly, compared with the control. Our study provides a new and relatively simple method for balancing the yield and the accumulation of antioxidants in basil production in plant factories with artificial lighting.

Supplementary Material

Supplementary Figure S2.1. Spectral distribution of the LED lamp used in the experiments.



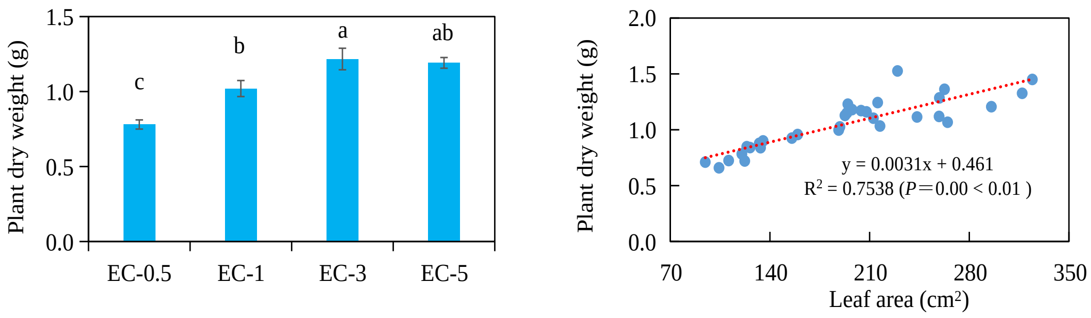
Supplementary Figure S2.2. Basil plants grown under 4 different EC values for 18 days after transplanting.



EC=0.5 EC=1.0

EC=3.0 EC=5.0

Supplementary Figure S2.3. The plant dry weight of basil under 4 different EC treatments at 34 days after sowing (A) and the scatter plots for the relationship between the plant dry weight (y) and the leaf area (x) (B).



(A) (B)

The plant dry weight = shoot dry weight + root dry weight. The error bars represent SEs (n = 8). Different lowercase letters represent significant differences among different EC treatments based on Tukey's test at $P < 0.05$.

Supplementary Table S2.1. Growth parameters of basil plants grown under four different EC values of the nutrient solution in experiment 1.

Growth parameter	Unit	Treatments				*p-value* [z]
		EC-0.5	EC-1	EC-3	EC-5	
Stem FW	(g plant^{-1})	1.78 ± 0.13 c[y]	2.85 ± 0.24 b	3.90 ± 0.35 a	2.68 ± 0.15 b	1×10^{-5}
Stem DW	(g plant^{-1})	0.16 ± 0.01 b	0.23 ± 0.02 a	0.27 ± 0.02 a	0.22 ± 0.01 ab	1×10^{-3}
Root FW	(g plant^{-1})	4.60 ± 0.22 b	5.12 ± 0.12 ab	5.81 ± 0.27 a	5.67 ± 0.17 ab	0.04
Root DW	(g plant^{-1})	0.22 ± 0.01 a	0.23 ± 0.01 a	0.29 ± 0.01 a	0.25 ± 0.01 a	0.07
plant height	(cm plant^{-1})	13.90 ± 0.35 c	17.15 ± 0.52 b	19.45 ± 0.51 a	17.30 ± 0.57 b	0.00
Stem diameter	(mm plant^{-1})	2.78 ± 0.09 b	3.33 ± 0.11 a	3.38 ± 0.14 a	2.79 ± 0.11 b	1.3×10^{-5}
Leaf number	(no. plant^{-1})	9.80 ± 0.67 b	11.63 ± 0.68 ab	14.38 ± 1.27 a	11.88 ± 0.48 ab	0.02

[z]The p-values for the ANOVA of the multiple mean comparison among the four EC treatments by Tukey's method applied to each row. [y]Means followed by the different letters are significantly different within a row, according to Tukey's test ($P < 0.05$). Each value is the mean ± SE of eight replicates.

Supplementary Table S2.2. The stomatal conductance (Gs), transpiration rate (Tr) and water use efficiency of photosynthesis (WUE_{Pn}) of basil leaves under 4 different EC treatments at 34 days after sowing in experiment 1.

Treatment Code	Gs (mol H_2O m^{-2} s^{-1})	Tr (mmol m^{-2} s^{-1})	WUE_{Pn} (mol H_2O·µmol^{-1} CO_2)
EC-0.5	0.119[y]	2.361	0.014
EC-1	0.136	2.186	0.018
EC-3	0.122	2.308	0.017
EC-5	0.118	2.342	0.015
ANOVA [z]	NS	NS	NS

[z]Results of analysis of variance (ANOVA) are shown. NS, non-significant. [y]Each value is the mean of four to six replicates.

Supplementary Table 2.3. Growth parameters of basil plants grown under different EC treatments in experiment 2.

Growth parameter	Unit	Treatments							*p-value* [z]
		5d-water	3d-water	5d-EC0.5	3d-EC0.5	5d-EC1	3d-EC1	Control	
Leaf area	(cm^2 $plant^{-1}$)	165.09 ± 17.92 c[y]	192.98 ± 14.86 bc	211.44 ± 14.12 b	220.21 ± 11.16 ab	224.71 ± 31.55 ab	211.55 ± 14.25 b	254.55 ± 32.70 a	0.02
Stem FW	(g $plant^{-1}$)	1.6 ± 0.16 c	2.39 ± 0.10 bc	3.2 ± 0.15 ab	2.82 ± 0.23 b	3.12 ± 0.16 ab	2.77 ± 0.28 b	3.90 ± 0.35 a	0.00
Stem DW		0.14 ± 0.01 bc	0.16 ± 0.01 bc	0.20 ± 0.01 bc	0.17 ± 0.01 bc	0.21 ± 0.01 ab	0.19 ± 0.02 bc	0.27 ± 0.02 a	7×10^{-6}
Root FW		5.2 ± 0.15 a	5.13 ± 0.20 a	4.93 ± 0.14 a	5.25 ± 0.22 a	5.13 ± 0.08 a	5.30 ± 0.15 a	5.23 ± 0.11 a	0.72
Root DW		0.26 ± 0.01 a	0.23 ± 0.01 ab	0.22 ± 0.01 a	0.22 ± 0.01 b	0.23 ± 0.01 ab	0.23 ± 0.01 ab	0.22 ± 0.01 b	0.01

[z]The p-values for the ANOVA of the multiple mean comparison among the four EC treatments by Tukey's method applied to each row. [y]Means followed by the different letters are significantly different within a row, according to Tukey's test ($P < 0.05$). Each value is the mean ± SE of eight replicates.

Supplementary Table S2.4. The percentage decrease in leaf fresh weight of basil compared with the control and the percentage increase in antioxidant capacity, total phenol contents (TPC), antioxidant capacity in leaves of the whole plant, TPC in leaves of the whole plant of basil compared with the control at 34 days after sowing in experiment 2.

Parameters	unit	Treatments					
		5d-Water	**3d-Water**	**5d-EC0.5**	3d-EC0.5	5d-EC1	3d-EC1
Leaf FW	%	24.4	**18.9**	**14.4**	16.5	12.0	10.7
Antioxidant capacity	%	172.6	**90.8**	**76.3**	49.5	12.7	32.7
TPC	%	235.4	**133.2**	**110.2**	98.3	43.8	77.3
Antioxidant capacity per plant	%	120.7	**67.4**	**66.0**	42.7	17.6	28.4
TPC per plant	%	194.4	**119.9**	**115.3**	101.8	62.9	95.3

CHAPTER 3. Growth and nutrient utilization in basil plant as affected by applied nutrient quantity in nutrient solution and light spectrum

3.1. Introduction

The world is increasingly facing the problem of resource shortage (Kozai, 2013), therefore it is particularly important to improve the resource use efficiency. In agricultural production, the excessive use of fertilizers is one of the factors limiting the sustainable development of agriculture (Lal, 2018). Hydroponics is an important vegetable cultivation method in protected agriculture, and the nutrient solution (NS) used in hydroponics can be recycled, which greatly improves the water and fertilizer use efficiency. However, the accumulation of root exudates of vegetable (Miller et al., 2020; Yu et al., 1993) in the recycled NS lead to the reduction of vegetable yield under long term recycled. Moreover, it is difficult to remove the root exudates. Therefore, the growers prefer to discard the recycled NS (Miller et al., 2020). The discharge of NS into the natural environment will cause environmental pollution and resource waste (Sago and Shigemura, 2018). Specifically, the discharge of a large amount of PO_4^{3-} into the water body leads to the eutrophication of the water body (Kumar et al., 2016; Karak et al., 2012). Besides, phosphate fertilizer waste will exacerbate phosphate rock depletion (Cordell et al., 2009). The nitrogen fertilizer in the NS mainly exists in the form of NO_3^- and NH_4^+ and the NH_4^+ is converted into nitrate ion by the nitrification of microorganisms in soil. These NO_3^- will enter the groundwater and cause excessive nitrate concentration, which is harmful to the human body (Savci, 2012). Moreover, catalytic ammonia synthesis consumes a lot of energy (Michalsky and Pfromm, 2012), which indicate that the ammonium waste causes a lot of energy waste. K^+ is an important source of groundwater salinization in semi-arid context (Buvaneshwari et al., 2020). Na^+ may cause soil particles to be dispersed and soil nutrient deficiency or imbalance, thereby affecting plant growth (Machado and Serralheiro, 2017). Therefore, it is of great significance to protect the environment and save resources by improving the absorption and utilization efficiency of nutrients.

The electrical conductivity (EC) of the NS can be easily measured, and by monitoring the EC of the nutrient solution, the total amount of available ions in the NS can be estimated (Trejo-Téllez and Gómez-Merino, 2012). Therefore, the EC management method is a common method to control the growth and quality of vegetables and herbs in hydroponics (SharathKumar et al., 2020). However, there are also some disadvantages of the EC management method. For example, the EC value does not reflect the specific ion content in the NS after a period using for plant cultivation (Miller et al., 2020). Moreover, long-term control of the EC at a target value may cause the disorder of the nutrients due to the selective absorption of nutrients by plants. On the one hand, vegetables often absorb certain nutrients (e.g., NO_3^-, PO_4^- and K^+) more than that they need under the NS of a constant EC (Maneejantra et al., 2016). The yield of the plant will no longer increase when the absorption of these nutrients exceeds a threshold (luxury absorption), which will result in the waste of resource (Maneejantra et al., 2016). On the other hand, $Ca^{2+}$and Mg^{2+} will be accumulated in the NS due to the relative weaker absorption capacity of plants. In addition, NS controlled by regulating EC value for a long time will cause accumulation of Na^+. Therefore, even if the EC value of the NS reaches the target value, the balance of the ions are no longer suitable for plant growth (Miller et al., 2020).

To compensate for the shortcomings of the EC management method, a new NS management method, quantitative nutrient management (QNM), was developed in recent years (Tsukagoshi et al., 2021). In QNM, the nutrients are supplied to the NS quantitatively and regularly, regardless of the EC value of the NS (Terabayashi et al., 2004; Tsukagoshi et al., 2015). Although QNM fluctuates the concentration of nutrients, the yield of lettuce is not significantly affected and most of the nutrients is absorbed (Sago and Shigemura, 2018). Moreover, QNM makes it possible to avoid luxury absorption of nutrients and increase the nutrient utilization by plants due to the nutrients can be supplemented according to the nutrient requirements of spinach (Maneejantra et al., 2016). Compared with the EC management method, the QNM is more beneficial to the future development of hydroponics in terms of saving resources and regulating vegetable quality. QNM has been applied to control the yield and quality of vegetables in recent years (Tsukagoshi et al., 2021; Sago and Shigemura, 2018).

The growth and nutrient absorption are significantly affected by ANQ in vegetables. The shoot dry weight of tomato decreased with the decrease of the ANQ (Matsuda et al., 2010). In another study, the leaf dry weight of tomato was not affected by different applied potassium amount and the potassium absorption increase with the increase of applied potassium amount (Tsukagoshi et al., 2021). Shoot fresh and dry weights of lettuce decreased with the decrease of applied nitrate amount and the nitrate absorption increase with the increase of applied nitrate amount (Sago and Shigemura, 2018).

A plant factory with artificial lighting (PFAL) is a closed system used to produce valuable plants such as vegetables and herbs (Pennisi et al., 2019a). Moreover, PFALs improve the nutrient, land and water use efficiency as compared to traditional agriculture (Graamans et al., 2018). The growth and nutrient absorption of vegetables can be not only controlled by nutrient supply but also by light spectrum in PFAL. The combination of red light and blue light is widely used as a light source for vegetable production in PFAL (Pennisi et al., 2019a; Song et al., 2020) and the effects of different R:B ratios on the growth and nutrient absorption of vegetables are significant. The shoot fresh weight of basil was higher at R:B ratios of 2:1, 3:1 and 4:1 than that at R:B ratios of 1:2 and 1:1 (Pennisi et al., 2019a); The shoot fresh and dry weights of lettuce were higher at R:B ratio of 3:1 than those at R:B ratios of 1:2, 1:1 and 2:1 (Pennisi et al., 2019b). The shoot fresh and dry weights of lettuce were highest at R:B ratio of 9:2 than those at other R:B ratios (Pinho et al., 2017). A R:B of 4:1 increased the concentration of Ca, Mg, P and S in the shoot of broccoli, however, the shoot fresh and dry weights were not affected by different R:B ratios (Kopsell et al., 2014).

These studies focused on the effects of nutrient supply and red light, blue light, or R:B ratios on the yield, nutrition concentration, and nutrition accumulation of different plants, but there are still no data on the effects of the combination of ANQ and R:B ratios on the total NA, NA efficiency (NAE), and NUE. From their results, we hypothesized that an R:B ratio between 2:1 and 4:1 could promote the yield of basil, which is one of the major herbs grown in PFALs. Different R:B ratios could also significantly affect the NA of macro-elements in NS, especially under different ANQs. In the present study, a low ANQ with an R:B ratio that maximizes yield is aimed to be determined to improve the NAE and NUE of basil plants in a PFAL. The results could expand our understanding of the association between nutrient uptake and light spectrum in basil plants and help the producers to produce basil more effectively and economically.

3.2. Materials and methods

3.2.1. Plant material and plant cultivation

Sweet basil (*Ocimum basilicum* L. var. *basilicum* L. cv. Genovese, Takii & Co., Ltd., Kyoto, Japan) seeds were sown in sponge cubes (2.3 × 2.3 × 2.8 cm, 14.8 cm^3) placed in germination boxes (22 × 14 × 5 cm, 1.54 L) at a temperature of 20 °C for 48 h for germination. The sponge cubes were soaked in an NS (N 22.20 mM, P 2.04 mM, K 9.12 mM, Mg 2.22 mM, Ca 4.92 mM, Fe 61.07 µM, Cu 0.38 µM, Zn 0.73 µM, Mo 0.25 µM, Mn 16.80 µM, and B 35.56 µM, under an EC of 3.0 dS m^{-1}) (Otsuka hydroponic composition, OAT Agrio Co., Ltd., Tokyo, Japan). The EC and pH of the NS were adjusted to 1.8 dS m^{-1} and 6.5, respectively. After germination, the seedlings were placed under white light-emitting diode (LED) lamps with a photosynthetic photon flux density (PPFD) of 200 ± 15 µmol m^{-2} s^{-1}. The spectrum of the white LED lamp is shown in Supplementary Figure S1. At 20 days after sowing, two experiments were conducted. Experiment 1 was to determine the daily NA of the basil plant under the optimal EC condition (EC = 3 dS m^{-1}) confirmed by our previous study (Ren et al., 2022) for designing the ANQ of the control in Experiment 2. Experiment 2 was to investigate the effects of different ANQ and R:B ratios on the growth and nutrient utilization of basil plants. Air pumps were used to supplement oxygen in both experiments.

In Experiment 1, the seedlings were transplanted to a cultivation box (32 × 18 × 9 cm, 5.18 L) containing 3 L NS (EC = 3 dS m^{-1}) till harvest. The NS volume and EC value of NS were adjusted to 3 L and 3 dS m^{-1}, respectively, every 2 days. Simultaneously, the pH was adjusted to 6.5. The plant density was 100 plants m^{-2}. The nutrient concentration was the same as described earlier. Light was provided using the LED lamps (GreenPower research modules, Philips, Pila, Poland) with a PPFD of 200 ± 15 µmol m^{-2} s^{-1} (R:B ratio of 7:3). The light spectrum is shown in Figure 1B. The temperature, photoperiod, CO_2 concentration, and relative humidity were set to 24 °C/21 °C (day/night), 16/8 h (day/night), 500 ppm, and 65–80%, respectively. The NS was sampled on the 10th, 15th, and 20th days after transplanting to determine the absorption of nutrients by the plants during 1–10, 11–15, and 16–20 days after transplanting.

In Experiment 2, the seedlings were subjected to 4×3 = 12 treatments in total. Two factors, namely, quantitative fertilization mode and light spectrum, were included in this experiment. For the quantitative fertilization mode, 4 levels of nutrient element quantity, 0.5, 1, 2, and 4 times the absorption quantity of nutrients determined in Experiment 1 (hereafter indicated by 0.5T, 1T, 2T, and 4T, respectively), were supplied during transplanting to harvest. The addition of nutrients was divided into three stages. The first stage was 1－10 days after transplanting, the second stage was 11－15 days after transplanting, and the third stage was 16－20 days after transplanting. The NS volume was adjusted to 3 L every 2 days, and simultaneously, the pH was adjusted to 6.5. The concentrated NS was added quantitatively on the 1st, 11th, and 16th days after transplanting. The fertilization design is shown in Table 1. For light spectra, 3 different R:B ratios, 3:7, 7:3, and 9:1 (hereafter indicated by RB3:7, RB7:3, and RB9:1, respectively), were used. The proportion of red light was calculated by defining the relative areas of the spectrum within the red light region (Piovene et al., 2015). The light was provided by dimmable red and blue LED lamps (GreenPower research modules, Philips, Pila, Poland). Six red LED lamps and six blue LED lamps were used in each treatment. The target R:B ratio was obtained by dimming the light intensity of red and blue LED lamps and was measured using a spectrum meter (Lighting Passport Pro, ALP-01, ASENSETEK INC., Taipei, Taiwan). Moreover, the total PPFD at the surface of planting panels was adjusted to 200±15 μmol m^{-2} s^{-1}. RB3:7, RB7:3, and RB9:1 indicate that the light intensities of red light and blue light were 60 and 140 μmol m^{-2} s^{-1}, 140 and 60 μmol m^{-2} s^{-1}, and 180 and 20 μmol m^{-2} s^{-1}, respectively. The treatment of 1T under RB7:3 was used as the control. The light spectra of different R:B ratios are shown in Figure 1. The temperature, photoperiod, CO_2 concentration, and relative humidity were set to 24 °C/21 °C (day/night), 16/8 h (day/night), 500 ppm, and 65－80%, respectively. From transplanting to harvest, the NS was sampled on the 10th, 15th, and 20th days to detect the contents of each nutrient element.

Figure 3.1. Spectral distribution of the LED lamps of RB3:7 (A) RB7:3 (B) and RB9:1 (C) used in the experiment.

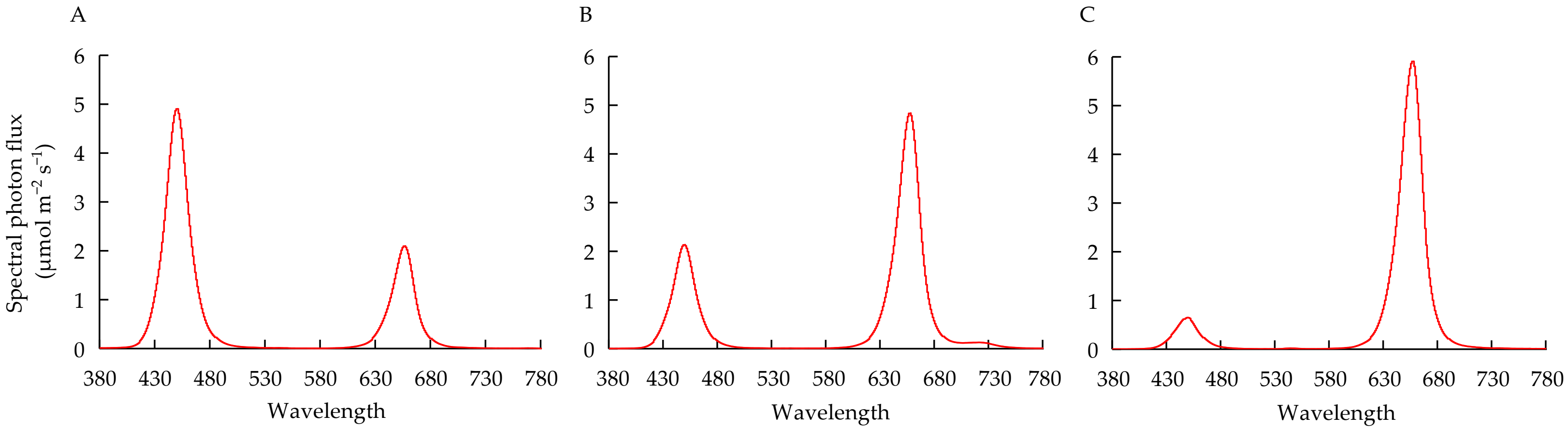


Table 3.1. Fertilization design for 1-20 days after transplanting.

Growth stage (days)	ANQ	Quantity of fertilizer supply (mg plant^{-1})								
		$Ca(NO_3)_2 \cdot 4H_2O$	KNO_3	$Mg(NO_3)_2 \cdot 6H_2O$	$NaNO_3$	K_2SO_4	NaH_2PO_4	KCl	$MgSO_4 \cdot 7H_2O$	KH_2PO_4
1-10 days	0.5T	33.50	36.23	19.22	24.00	10.45	14.57	6.17	0.00	0.00
	1T	67.00	72.46	38.44	48.00	20.90	29.13	12.33	0.00	0.00
	2T	134.00	144.91	76.88	96.00	41.81	58.27	24.67	0.00	0.00
	4T	268.00	289.82	153.75	192.00	83.61	116.53	49.33	0.00	0.00
11-15 days	0.5T	51.24	54.76	3.20	12.75	0.00	0.00	0.00	19.12	15.61
	1T	102.47	109.53	6.41	25.50	0.00	0.00	0.00	38.24	31.23
	2T	204.94	219.05	12.81	51.00	0.00	0.00	0.00	76.48	62.46
	4T	409.89	438.1	25.63	102.00	0.00	0.00	0.00	152.95	124.91
16-20 days	0.5T	126.12	80.04	16.02	6.38	0.00	0.00	0.00	30.80	32.37
	1T	252.24	160.08	32.03	12.75	0.00	0.00	0.00	61.61	64.74
	2T	504.48	320.15	64.06	25.5	0.00	0.00	0.00	123.21	129.48
	4T	1008.95	640.31	128.13	51.00	0.00	0.00	0.00	246.42	258.96

0.5T, 1T, 2T and 4T represent the 4 levels of nutrient element amount, 0.5 times, 1 time, 2 times and 4 times of the amount of nutrients absorbed by basil plant from transplanting to harvest determined by experiment 1.

3.2.2. Measurements

3.2.2.1. Plant growth parameters

The shoot, leaf, stem, and root fresh weights of sweet basil were measured on the harvesting day. Then, the sweet basil was oven-dried at 80°C for 3 days to a constant weight for dry weights measurement. Six replicates were performed for each treatment.

Total leaf area was measured with a leaf area meter (Li–3000, Li–Cor, Lincoln, NE, USA). LMA was determined as leaf dry weight divided by leaf area. Six replicates were performed for each treatment.

3.2.2.2. Gas-Exchange parameters

At 20 days after transplanting, the Gas-exchange parameters were determined with a gas exchange system (LI-6400, Li-Cor, Inc., Lincoln, NE, USA) and the integrated fluorescence chamber head (LI-6400-40, Li-Cor, Inc., Lincoln, NE, USA) and the third pair of leaves from the top were used for the measurement of each parameter. Three replicates were performed for each treatment. PPFD, CO_2 concentration, relative humidity and air temperature inside the leaf chamber were set at 200 μmol m^{-2} s^{-1}, 500 mmol mol^{-1}, 65% and 22℃, respectively. The R:B ratio during measurement is also set to be consistent with the actual R:B ratio during growth. The R:B ratios during measurement were set to 3:7, 7:3, and 9:1, respectively.

3.2.2.3. Chlorophyll fluorescence parameters

To evaluate the light absorption, transfer, dissipation, and distribution in the photosystem of basil under different treatments, chlorophyll fluorescence parameters were measured with a gas exchange system (LI-6400, Li-Cor, Inc., Lincoln, NE, USA) and the integrated fluorescence chamber head (LI-6400-40, Li-Cor, Inc., Lincoln, NE, USA). After a dark period of one night, the maximum chlorophyll fluorescence yield (F_m) and the minimum chlorophyll fluorescence yield (F_o) were measured before the LED light is turned on. The maximum quantum yield of the PSII primary photochemistry (Fv/Fm) was calculated as (Fm–F_o)/Fm. After the F_m and F_o were determined, the same leaves are exposed to actinic light for light adaptation. Then the steady state chlorophyll fluorescence level (Fs), the minimum chlorophyll fluorescence yield (F'_o), the maximum chlorophyll fluorescence yield (F'_m) were measured of the light-adapted leaves. The quantum yield of PSII electron transport (PhiPSII), the efficiency of excitation energy capture by open PSII reaction centers (F'_v/F'_m), photochemical quenching (qP), non-photochemical quenching (qN) and the electron transport rate (ETR) were calculated as follows:

PhiPSII=($F'_m - F$s)/F'_m; F'_v/F'_m=($F'_m - F'_o$)/F'_m; qP= ($F'_m - F$s)/($F'_m - F'_o$); qN=(Fm $- F'_m$)/(Fm $- F'_o$); ETR = 0.5 × absI × PhiPSII, where 0.5 is the fraction of absorbed light reaching photosystem II, absI is absorbed irradiance taken as 0.85 of incident irradiance.

3.2.2.4 Nutrient absorption (NA), nutrient use efficiency (NUE) of shoot dry weight, nutrient absorption efficiency (NAE), nutrient waste (NW) from producing 1 g of shoot dry weight.

The nutrient content was measured using an ion chromatography system (ICS-1100, Thermo Fisher Scientific, Inc., Japan).

The NA, NUE, NAE, and NW were calculated as follows:

NA = TAN − FAN.

where TAN is the total amount of applied nutrient and FAN is the final amount of nutrients remaining.

NUE = SDW/NA (Dobermann, 2007).

where SDW is the shoot dry weight.

NAE = NA/TAN (Hoshi et al., 1999).

NW = FAN/SDW.

3.2.3. Statistical analysis

The data were subjected to analysis of variance and the means were compared between treatments using Tukey’s test in SPSS statistical software (IBM SPSS Statistics,Version 19.0. Armonk, NY, USA: IBM Corp.). A p-value < 0.05 was considered significant.

3.3. Results

3.3.1. Experiment 1:

3.3.1.1. The daily absorption of nutrients by basil plants

The daily absorption of NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} by basil plants increased with the stage of the plant. Basil plants had the largest daily absorption of NO_3^-, followed by K^+, PO_4^{3-}, Ca^{2+}, SO_4^{2-}, Mg^{2+} (**Table 2**).

Table 3.2 Daily absorption of NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} by basil plants at 1-10 days, 11-15 days, 16-20 days and 1-20 days after transplanting in experiment 1.

Growth stag	Days	Unit	Nutrients					
			NO_3^-	PO_4^{3-}	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}
Stage 1	1-10	mg $plant^{-1}$ day^{-1}	13.33	2.31	4.38	1.12	0.36	1.14
Stage 2	11-15		28.60	4.35	10.32	3.50	0.86	2.86
Stage 3	16-20		50.97	9.01	16.01	8.57	1.78	4.69
Avereage	1-20		25.56	4.50	8.77	3.58	0.84	2.45

3.3.2. Experiment 2:

3.3.2.1. Comparison of plant growth between experiment 1 and the control group (1T under RB7:3) in experiment 2.

There was no significant difference in the shoot and leaf fresh weights, and shoot and leaf dry weights of the basil plants between experiment 1 and the control group in experiment 2.

Table 3.3. Shoot and leaf fresh weights and shoot and leaf dry weights of basil plants in experiment 1 and the control group (1T under RB7:3) in experiment 2.

Growth parameters	Experiment 1	The control group in experiment 2
Shoot fresh weight	16.08 a[z]	16.02 a
Leaf fresh weight	12.50 a	12.16 a
Shoot dry weight	1.30 a	1.16 a
Leaf dry weight	1.00 a	0.89 a

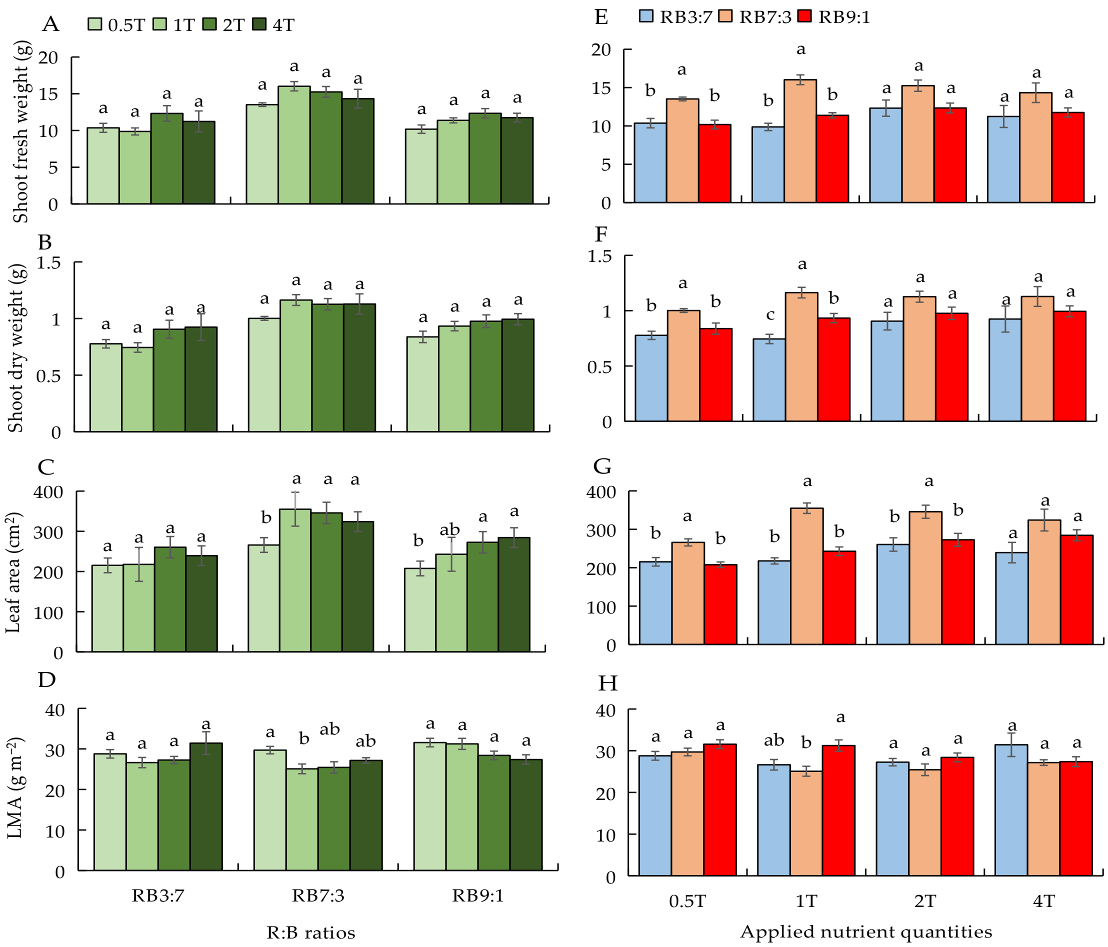
[z] Data are shown as means (n = 6). The same letters in each row indicate no significant differences between the experiment 1 and the control group of experiment 2 at $p < 0.05$, determined by T-test.

3.3.2.2. Plant growth

To describe the results more clearly, we used the same data to plot two types of graphs in different forms (left and right). As observed in the left side of Figure 3.2, there was no difference in all growth parameters at different ANQ treatments under RB3:7 (Figure 3.2A－D). Under RB7:3, the shoot fresh and dry weights were not affected by different ANQ treatments; however, the leaf area at 0.5T was significantly lower than that at other ANQ treatments. The LMA at 0.5T was significantly higher than that at 1T, but there was no significant difference in LMA among 1T, 2T, and 4T (Figure 3.2A－D). Under RB9:1, the shoot fresh and dry weights were not significantly different among different ANQ treatments. The leaf area was lower at 0.5T than at 2T and 4T. The difference in LMA under different ANQ treatments was not significant (Figure 3.2A－D).

As observed in the right side of Figure 2, under 0.5T, the shoot fresh and dry weights and leaf area were significantly higher at RB7:3 than at RB3:7 and RB9:1 (Figure 3.2E－G); however, the differences in LMA at different R:B ratios were not significant (Figure 3.2H). The shoot fresh and dry weights and leaf area at different R:B ratios under 1T exhibited a similar trend as those parameters at different R:B ratios under 0.5T; however, the LMA at RB9:1 was significantly higher than that at RB7:3 (Figure 3.2E－H). Under 2T, the leaf area at RB7:3 was significantly higher than that at RB3:7 and RB9:1, but the other growth parameters showed no significant differences among different R:B ratios (Figure 3.2E－H). No significant difference was observed in all parameters among different R:B ratio treatments under 4T (Figure 3.2E－H).

Figure 3. 2. Shoot fresh (A; E) and dry weights (B; F), leaf area (C; G), and leaf dry mass per area (LMA) (D; H) at 20 days after transplanting under different ANQ treatments and R:B ratios.



0.5T, 1T, 2T, and 4T represent 4 different ANQ treatments, respectively. RB3:7, RB7:3, and RB9:1 represent R:B ratios of 3:7, 7:3, and 9:1, respectively. The error bars represent SEs (n = 6). Based on Tukey's test at $p < 0.05$. Different lowercase letters represent significant differences among different treatments.

3.3.2.3. Gas-exchange and chlorophyll fluorescence parameters

Net photosynthetic rate, transpiration rate, electron transport rate, maximum quantum yield of PSII primary photochemistry, efficiency of excitation energy captured by open PSII reaction centers, quantum yield of PSII electron transport, photochemical quenching, and the nonphotochemical quenching were not affected by the ANQ treatments and R:B ratios (Table 3.4).

Table 3.4. Photosynthesis parameters of basil plants grown under different ANQ treatments and R:B ratios.

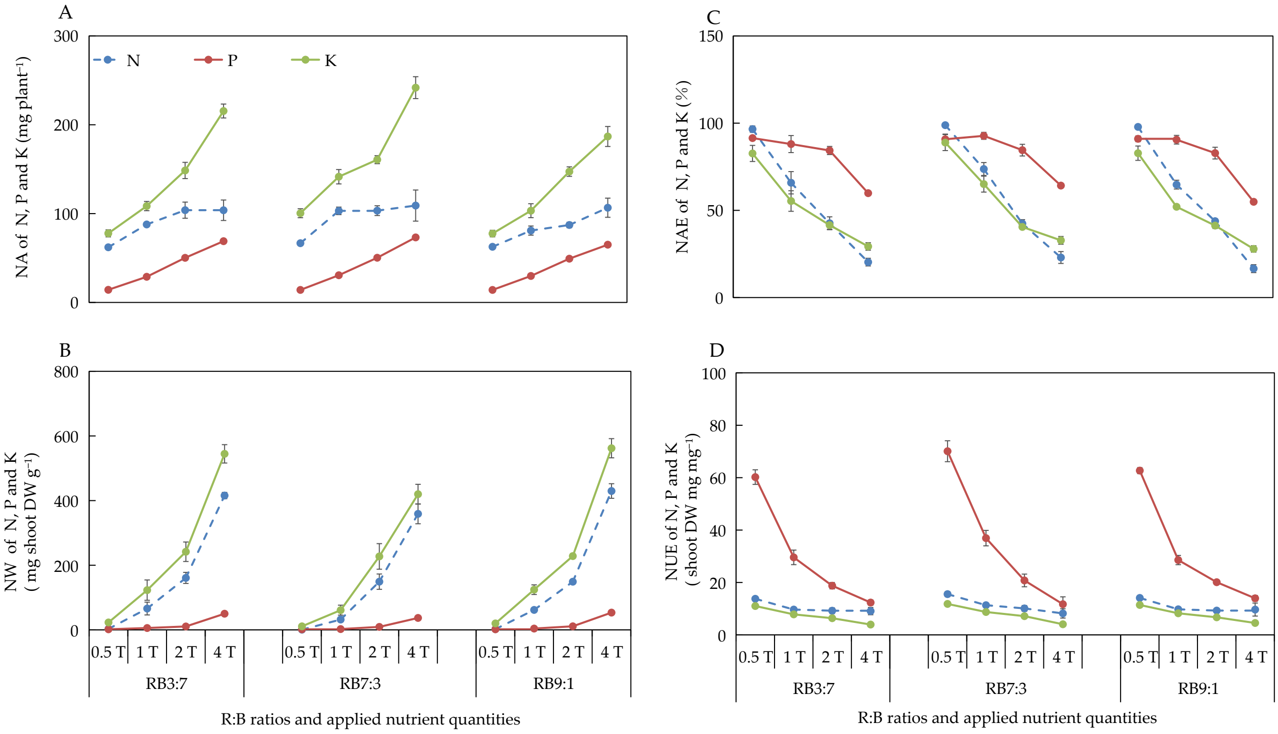
Photosynthesis	Unit	ANQ	R:B ratios					
			RB3:7		RB7:3		RB9:1	
Net photosynthetic rate	(μmol CO_2 m^{-2} s^{-1})	0.5T	9.76	±0.70[z]	10.32	±1.04	8.99	±0.86
		1T	10.70	±0.30	8.59	±0.90	8.19	±1.68
		2T	11.37	±0.85	9.79	±1.67	8.49	±0.70
		4T	10.00	±0.99	9.66	±1.34	8.73	±0.88
Tr	(mmol m^{-2} s^{-1})	0.5T	1.58	±0.18	1.48	±0.28	1.79	±0.08
		1T	1.47	±0.33	1.48	±0.05	2.08	±0.12
		2T	1.91	±0.18	1.55	±0.07	1.78	±0.18
		4T	1.73	±0.16	1.56	±0.25	1.36	±0.23
ETR	(μmol electrons m^{-2} s^{-1})	0.5T	44.10	±4.04	44.57	±7.33	45.47	±3.21
		1T	44.47	±4.32	37.19	±6.65	39.87	±0.97
		2T	43.19	±3.23	40.59	±3.59	41.61	±5.30
		4T	43.74	±4.69	37.81	±8.26	35.06	±5.81
Fv/Fm		0.5T	0.82	±0.01	0.81	±0.005	0.80	±0.02
		1T	0.82	±0.02	0.81	±0.008	0.80	±0.004
		2T	0.82	±0.01	0.81	±0.009	0.80	±0.01
		4T	0.80	±0.03	0.81	±0.013	0.80	±0.001
Fv'/Fm'		0.5T	0.69	±0.02	0.68	±0.07	0.68	±0.04
		1T	0.69	±0.04	0.66	±0.06	0.68	±0.003
		2T	0.67	±0.03	0.69	±0.02	0.70	±0.01
		4T	0.70	±0.02	0.64	±0.05	0.63	±0.02
PhiPSII		0.5T	0.56	±0.05	0.51	±0.11	0.58	±0.04
		1T	0.57	±0.05	0.48	±0.08	0.51	±0.01
		2T	0.55	±0.04	0.52	±0.05	0.53	±0.07
		4T	0.64	±0.01	0.48	±0.11	0.45	±0.07
qP		0.5T	0.81	±0.05	0.82	±0.09	0.85	±0.03
		1T	0.82	±0.04	0.72	±0.06	0.75	±0.02
		2T	0.83	±0.03	0.75	±0.05	0.76	±0.11
		4T	0.89	±0.02	0.75	±0.11	0.71	±0.13
qN		0.5T	0.52	±0.11	0.52	±0.14	0.53	±0.09
		1T	0.52	±0.11	0.61	±0.09	0.55	±0.08
		2T	0.55	±0.09	0.57	±0.02	0.53	±0.02
		4T	0.45	±0.02	0.56	±0.13	0.64	±0.04

Net photosynthetic rate, transpiration rate (Tr), electron transport rate (ETR), maximum quantum yield of PSII primary photochemistry (Fv/Fm), efficiency of excitation energy captured by open PSII reaction centers (F′v/F′m), quantum yield of PSII electron transport (PhiPSII), photochemical quenching (qP), and the nonphotochemical quenching (qN) of basil plants at 20 days after transplanting under different ANQ treatments and R:B ratios. 0.5T, 1T, 2T, and 4T represent four different ANQ treatments. RB3:7, RB7:3, and RB9:1 represent R:B ratios of 7:3, 3:7, and 9:1, respectively. [z]Each value is the mean ± SE of three replicates.

3.3.2.4. Absorption and utilization of N, P and K under different ANQ treatments

The different ANQ treatments exerted a significant effect on the absorption and utilization of N, P, and K, irrespective of the R:B ratios. Furthermore, the absorption and utilization of N, P, and K by the basil plant showed a clear trend under different ANQ treatments, irrespective of the R:B ratios. Specifically, the NA and NW of N, P, and K increased with the increase of ANQ (Figure 3.3A, B). The NAE and NUE of N, P, and K decreased with the increase of ANQ (Figure 3.3C, D).

Fig. 3.3. Nutrient absorption (NA) (A), nutrient waste (NW) (B) from producing 1 g shoot dry weight, nutrient absorb efficiency (NAE) (C), and nutrient use efficiency (NUE) (D) of shoot dry weight of N, P, and K after 20 days of cultivation under different ANQ treatments and R:B ratios.

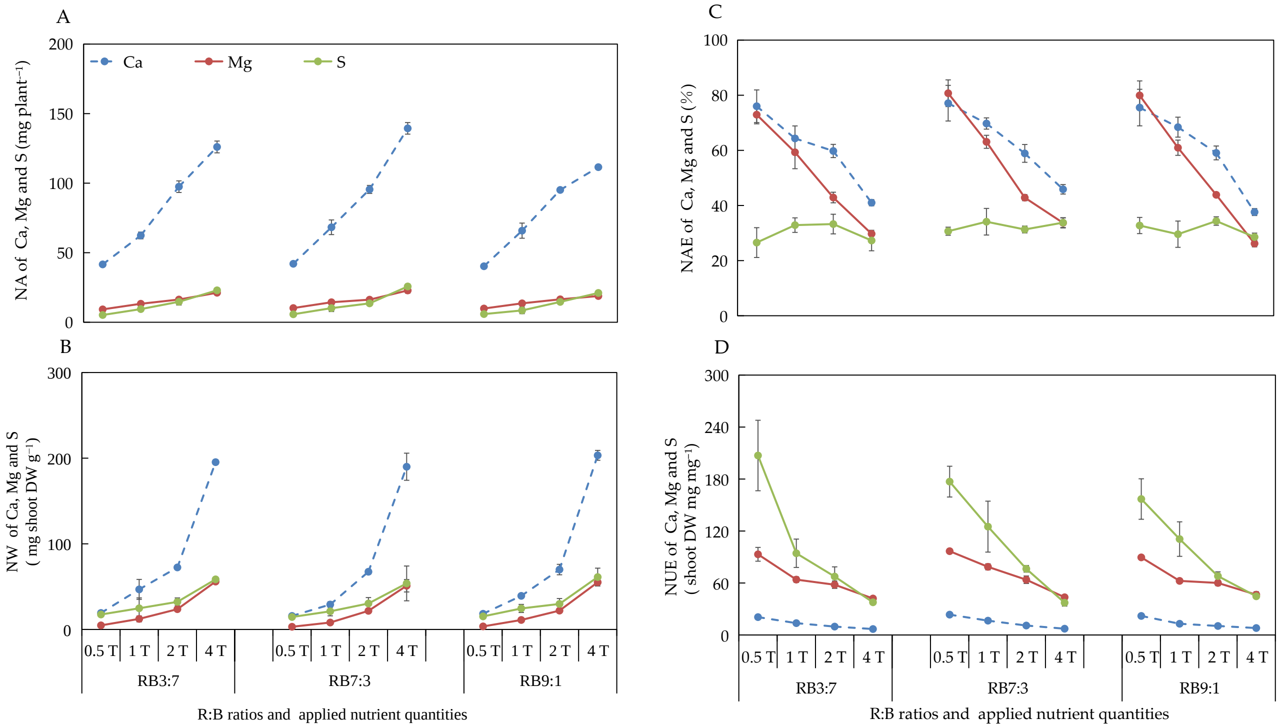


0.5T, 1T, 2T, and 4T represent 4 different ANQ treatments. RB3:7, RB7:3, and RB9:1 represent the R:B ratios of 7:3, 3:7, and 9:1, respectively. The error bars represent SEs (n = 3).

3.3.2.5. Absorption and utilization of Ca, Mg and S under different ANQ treatments

The effect of different ANQ treatments on the absorption and utilization of Ca, Mg and S was consistent with that of N, P, and K, with the only difference being the effect on the NAE of S. The different ANQ treatments exerted no significant effect on the NAE of S, irrespective of the R:B ratios.

Fig. 3.4. Nutrient absorption (NA) (A), nutrient waste (NW) (B) from producing 1 g shoot dry weight, nutrient absorb efficiency (NAE) (C), and nutrient use efficiency (NUE) (D) of shoot dry weight of Ca, Mg, and S after 20 days of cultivation under different ANQ treatments and R:B ratios.



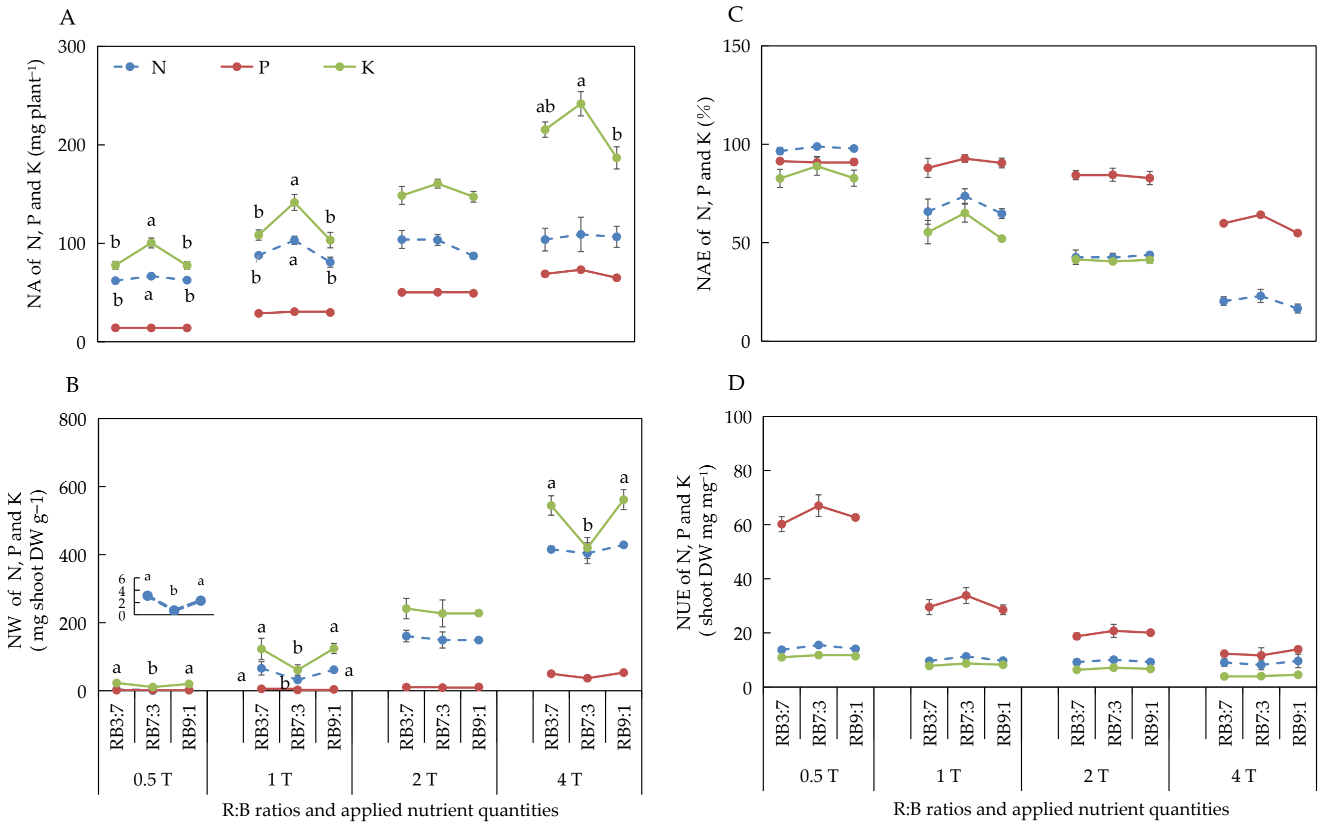
0.5T, 1T, 2T, and 4T represent 4 different ANQ treatments. RB3:7, RB7:3, and RB9:1 represent the R:B ratios of 7:3, 3:7, and 9:1, respectively. The error bars represent SEs (n = 3).

3.3.2.6 Absorption and utilization of N, P, and K at different R:B ratios

The NA of N and K was significantly increased at RB7:3 compared with that at other R:B ratios under 0.5T and 1T. However, the NA of N and K remained unaffected at different R:B ratios under 2T. The NA of N remained unaffected at different R:B ratios under 4T. However, the NA of K was significantly higher at RB7:3 than at RB9:1, but there was no significant difference in the NA of K between RB3:7 and RB7:3 under 4T. There was also no significant difference in the NA of P among different R:B ratios, irrespective of the ANQ treatments.

The NW of N and K was significantly decreased at RB7:3 compared with that at other R:B ratios under 0.5T and 1T. However, these parameters were not affected at different R:B ratios under 2T. Furthermore, the NW of N remained unaffected at different R:B ratios under 4T. The NW of K was significantly lower at RB7:3 than at other R:B ratios under 4T. No significant difference was observed in the NW of P among different R:B ratios, irrespective of the ANQ treatments. The NAE and NUE of N, P, and K were not affected at different R:B ratios, irrespective of the ANQ treatments.

Fig. 3.5 Nutrient absorption (NA) (A), nutrient waste (NW) (B) from producing 1 g shoot dry weight, nutrient absorb efficiency (NAE) (C), and nutrient use efficiency (NUE) (D) of shoot dry weight of N, P, and K after 20 days of cultivation under different ANQ treatments and R:B ratios.



0.5T, 1T, 2T, and 4T represent 4 different ANQ treatments. RB3:7, RB7:3, and RB9:1 represent the R:B ratios of 7:3, 3:7, and 9:1, respectively. The small graph in Figure 5B represents the NW of N at different R:B ratios under 0.5T. The error bars represent SEs (n = 3). On the basis of Tukey's test at $p < 0.05$. Different lowercase letters represent significant differences among different treatments.

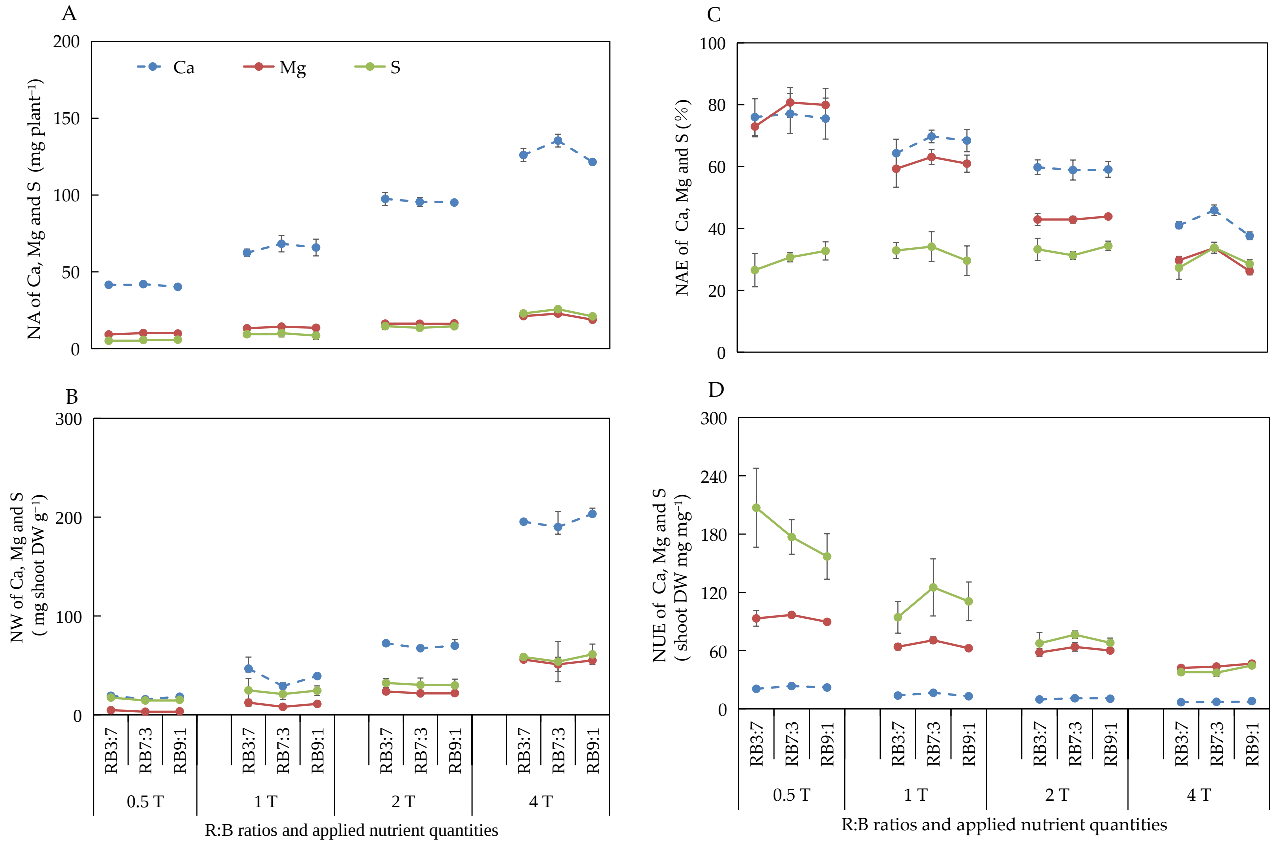
3.3.2.7 Absorption and utilization of Ca, Mg, and S at different R:B ratios

The NA of Ca tended to increase with the increase of the R:B ratios from 3:7 to 7:3 and then decrease when the R:B ratios increased from 7:3 to 9:1 under 1T and 4T. The NW of Ca showed the opposite tendency of NA under the same treatments. There were no obvious changes in the NA and NW of Ca at different R:B ratios under 0.5T and 2T. Moreover, there were no obvious changes observed in the NA and NW of Mg and S at different R:B ratios, irrespective of the ANQ treatments (Figure 3.6A,B).

The NAE of Mg tended to increase with the increase of the R:B ratios from 3:7 to 7:3 and then decrease when the R:B ratios increased from 7:3 to 9:1 under 0.5T, 1T, and 4T. The changes of NAE in Ca showed similar trends as Mg under 1T and 4T. The NAE of S showed different patterns with the change in the R:B ratios under different ANQ treatments, whereas there were no significant differences observed. There were no changes in the NAE of Ca, Mg, and S at different R:B ratios under 2T (Figure 3.6C).

The NUE of S showed a decreasing trend with the increase of R:B ratios under 0.5T, whereas it tended to first increase and then decrease with the increase of R:B ratios under 1T. Moreover, the NUE of Ca and Mg were not affected at different R:B ratios, irrespective of the ANQ treatments (Figure 3.6D).

Fig. 3.6 Nutrient absorption (NA) (A), nutrient waste (NW) (B) from producing 1 g shoot dry weight, nutrient absorb efficiency (NAE) (C), and nutrient use efficiency (NUE) (D) of shoot dry weight of Ca, Mg, and S after 20 days of cultivation under different ANQ treatments and R:B ratios.



0.5T, 1T, 2T, and 4T represent 4 different ANQ treatments. RB3:7, RB7:3, and RB9:1 represent the R:B ratios of 7:3, 3:7, and 9:1, respectively. The error bars represent SEs (n = 3).

3.4. Discussion

3.4.1. Growth response of basil to ANQ and R:B ratios.

The shoot fresh and dry weights of basil were not affected significantly by the different ANQ regardless of the R:B ratios in the present study (Figure 2E, F). This result indicates that compared with the higher ANQ (1T, 2T and 4T), the lower ANQ (0.5T) had met the nutrient requirements to produce the same yield of basil. In our study, ANQ (1T) is the amount of nutrients absorbed by basil at the optimal EC (EC=3.0 dS m^{-1}). Moreover, there was no significant difference of the shoot fresh and dry weights, leaf fresh and dry weights between the EC-management and quantitative nutrient-management (QNM) (Table 3.3). Which indicated that the ANQ (1T) had met the nutrient need of basil growth. When the amount of nutrient absorbed by plants exceeds a threshold (luxury absorption), its yield no longer increased with the increase in nutrient absorption. For instance, with the increase of EC of nutrient solution, the absorption of some macronutrients (N, P, K, Ca) by lettuce increased, but the yield of lettuce did not increase (Samarakoon et al., 2006). In our study, although the nutrient absorption increased as the ANQ increased from 1T to 4T, the shoot fresh and dry weights no longer increased. Which indicated that the ANQ of 2T and 4T may cause the luxury absorption of nutrients by basil. Besides, insufficient nutrients can lead to nutrient deficiency that limit plant growth and development (Zeng et al., 2014). In the present study, the ANQ of 0.5 T did not significantly decrease the yield of basil as compared to the ANQ of 1T, which indicated that the ANQ of 0.5 T did not cause nutrient deficiency for basil growth.

In the present study, the shoot fresh and dry weights of basil were significantly higher at RB7:3 than those at RB3:7 and RB9:1 under the ANQ of 0.5T and 1T. In addition, there was no significant difference in shoot fresh and dry weights among different R:B ratios at 2T and 4T. which indicates that the response of basil growth to the same R:B ratios was regulated by different ANQ treatments. Different R:B ratios produced differences in basil biomass at 0.5T and 1T in our study. However, the net photosynthetic rate and chlorophyll fluorescence parameters were not affected by different R:B ratios (Table 3.4). The photosynthetic rate in basil and strawberry (blue light percentage from 7.3 to 37.7%) (Piovene et al., 2015) and the chlorophyll fluorescence parameters (Fv/Fm, PhiPSII, and Fv/Fm-PhiPSII) in cucumber (blue light percentage from 0 to 100%) (Hogewoning et al., 2010) were significantly affected by R:B ratios when the proportion of blue light was below 10%. In the present study, the proportion of blue light is equal to or greater than 10%, which may be a possible factor leading to these results. Moreover, the leaf area was significantly higher at RB7:3 than at RB3:7 and RB9:1 under 0.5T and 1T (Figure 2G), and a significant positive correlation between shoot dry weight and leaf area was also found (Table S2). Hence, different R:B ratios influenced the leaf area of the basil plant, and thus, the photoassimilates of the entire plant were influenced. Another study also reported that different light spectra affected the interception of light by affecting the leaf area of green basil, resulting in different yields (Lin et al., 2021). Besides, it is reported that the fluorescence Fv/Fm of 3 basil cultivars was not affected by different electrical conductivities (ECs) of 1.0, 2.0, and 3.0 dS m^{-1} (Ciriello et al., 2020), and the photosynthetic rate of basil was not influenced by different ECs of 0.5, 1.0, 3.0, and 5.0 dS m^{-1} (Ren et al., 2022), indicating that the photosynthesis of basil is not easily affected by the nutrient amount in the nutrient solution in the ranges used. Moreover, N and P are considered very important for photosynthesis because photosynthesis is a highly regulated process that is coordinately operative with N metabolism (Iglesias et al., 2005) on the one hand. On the other hand, carbon is fixed through the photosynthetic carbon reduction cycle in the chloroplast, and the fixed carbon combines with the phosphate as triose phosphate (triose-P) in the cytosol, then the triose-P is converted to sucrose (Rychter et al., 2005). Therefore, photosynthesis would be affected if N and P supplies were not enough for plants. The photosynthesis parameters were not affected by different ANQs, which indicated that there was no N and P deficiency for basil plants in the ranges used in the present study.

3.4.2. Nutrient absorption and utilization efficiency to ANQ and R:B ratios.

Different ions have different transport mechanisms. For example, the uptake of NO_3^-, PO_4^-, and SO_4^{2-} is driven by cotransport with H^+. The uptake of Ca^{2+} and K^+ occurs via channels (transmembrane proteins) (Reid et al., 2003). Although the transport mechanisms are different for different ions, the electrochemical potential gradient is the driving force for ion transport (Reid et al., 2003; Yang et al., 2015). Differences in ion concentration inside and outside root cells create different electrochemical potential gradients (Taiz et al., 2015). High ANQ could result in high ion concentration and high electrochemical potential gradients, and, thus, high ANQ promoted nutrient absorption in the present study (Figures 3A and 4A). In general, the absorption of ions by plants is mainly affected by two factors, ion concentration and the number of transport proteins in the roots (Li, 2002). It usually increases with increasing ion concentration firstly and stops increasing when the ion concentration reaches a certain high level due to the limitation of the number of transport proteins on the root cell membrane. In the present study, the absorption of ions by basil plants kept increasing with the increase in ANQ under all light conditions, indicating that the ion concentration was the main factor responsible for the increase in ion absorption.

In our study, the absorption of N, P, K, Ca, Mg and S by basil plants increased with the increase of ANQ at all of the three R:B ratios. However, the shoot fresh and dry weights did not increase with the increase of ANQ in our study. Similarly, the absorption of K by tomato increases as the ANQ of K increases in hydroponic cultivation and the leaf dry weight did not increase with the increased absorption of K (Tsukagoshi et al., 2021). The absorption of excess nutrients that cannot be converted into economic crop yields is called luxury absorption (Maneejantra et al., 2016) and luxury absorption will cause the waste of nutrients. In this study, the 0.5T minimize the nutrient absorption without sacrificing yield of basil, which greatly reduces the wastage of nutrients due to luxury absorption. Besides, although the nutrient absorption by basil plants increased with the increase of ANQ, the increase in the rate of nutrient absorption lower than the rate of increase of ANQ, which resulted in a decrease in the AUE of basil plants for N, P, K, Ca and Mg with increasing ANQ and an increase of nutrients remaining in the nutrient solution after the basil is harvested with increasing ANQ.

In PFAL, NS recycling is often used to reduce nutrient waste and environmental pollution (Nederhoff and Stanghellini, 2010). However, the recycled NS often results in crop yield reduction and many planters prefer to discard the circulating NS (Miller et al., 2020). The discharge of nutrients into the environment will cause a series of environmental problems, such as eutrophication of surface waters and coastal marine ecosystems, nitrate pollution of groundwater, the increase of the greenhouse gas concentration (nitrous oxide) and the development of photochemical smog etc. (Ni et al., 2011,Vitousek et al., 2009,Ju et al., 2009). In our study, a ANQ of 0.5T minimized the amount of NO_3^-, PO_4^{3-}, K^+, Ca^{2+} and Mg^{2+} remaining in the nutrient solution as compared to other ANQ, which greatly reduced the waste of nutrients and environmental pollution.

NUE represents the plant's ability to convert absorbed nutrients into biomass (Siddiqi and Glass, 1981) and there are three ways to improve NUE: 1. The yield is unchanged and the absorption of nutrients is reduced. 2. Yield increases, nutrient absorption remains unchanged. 3. The increase in the rate of biomass more than the rate of increase of absorption of nutrients (An et al., 2005). In the present study, the shoot fresh and dry weights of basil plant was not significantly affected by different ANQ and the absorption of N, P, K, Ca, Mg and S was reduced with the decrease of the ANQ, which result in the NUE of N, P, K, Ca, Mg and S increased with the decrease of the ANQ.

In this study, the shoot fresh and dry weights of basil did not increase with the ANQ increase and the remaining amount of nutrients in the NS after harvest increases with the increase of ANQ, which results in an increase in NW of N, P, K, Ca, Mg and S with increased ANQ. The nutrient absorption and utilization efficiency were not significantly affected by different R:B ratios regardless of the ANQ in our study.

There were significant differences in the NA and NW of N and K among different R:B ratios in the present study. The transpiration rate was not affected by different R:B ratios (Table 3.4). However, the leaf area of basil plant was significantly higher at RB7:3 than at other R:B ratios under 0.5T and 1T, which led to higher transpirational fluxes of the entire basil plant at RB7:3 than at other R:B ratios. The increased transpirational fluxes may be the reason for the high N and K absorption in the present study, because NA increased with the increase of transpirational fluxes in wheat (Houshmandfar et al., 2018). Regarding the NW of N and K, the NA was higher at RB7:3 than at other R:B ratios under 0.5T and 1T, which led to the FAN in the solution tank after the harvest was lower at RB7:3 than at other R:B ratios. Meanwhile, the shoot dry weight was significantly higher at RB7:3 than at other R:B ratios under 0.5T and 1T. The decreased FAN and the increased shoot dry weight led to a significant decrease in the NW of N and K at RB7:3 compared with that at other R:B ratios under 0.5T and 1T.

3.5. Conclusion

This study explored the effects of quantitative nutrient management with different applied nutrient quantities and red:blue ratios on the growth and nutrient utilization of basil plant. Results showed that low applied nutrient quantity significantly increased the nutrient use efficiency and nutrient absorption efficiency and decreased the nutrient waste without decreasing the shoot fresh and dry weights of basil plant. Furthermore, we observed that RB7:3 was more conducive to basil production than other red:blue ratios under a low applied nutrient quantity condition because RB7:3 promoted plant growth and nutrient absorption of N and K and decreased the nutrient waste of N and K. Therefore, quantitative nutrient management combined with an optimal red:blue ratio could enhance the growth and nutrient utilization of basil plant and reduce resource waste in hydroponic vegetable production in a plant factory with artificial lighting. The quality of basil plant was not evaluated in the present study. Further studies are required to investigate the effect of the combination of quantitative nutrient management and red:blue ratios on the quality of basil plant in the future.

CHAPTER 4. Accumulation of secondary metabolites and nitrate content in basil as affected by light spectrum and applied nutrient amount in nutrient solution

4.1. Introduction

As an essential oil crop and culinary herb, basil (*Ocimum basilicum* L.) is grown worldwide (Hossain et al., 2010). For growers, in addition to yield, quality is also an important indicator to measure the economic value of basil. Basil is rich in antioxidants, reducing the risk of some human diseases (Filip et al., 2016). Antioxidant compounds, mainly phenolic substances (Gonçalves et al., 2013), synthesized by plants as secondary metabolites, act in the defense mechanism of plants against reactive oxygen species, preventing oxidative damage. Moreover, the antioxidant capacity of plants is well related to the total phenolic content (TPC) (Kaur et al., 2002). Therefore, increasing the antioxidant accumulation, such as TPC, can improve basil quality. The antioxidants of basil are regulated by environmental conditions (light, nutrient solution, etc.) (Dou et al., 2019a; Ren et al., 2022). Besides this, environmental conditions, such as light quality, light intensity, nutrient solution, temperature, and carbon dioxide concentration, can be controlled in plant factories with artificial lighting (PFAL). Therefore, many growers begin to use PFAL to produce high-quality basil.

Vegetable quality is affected by integrated environmental conditions such as light, nutrient supply (Song et al., 2020). Vegetable quality can be regulated by light quality (Dou et al., 2019b), applied nutrient amount (Sago et al., 2018), and light intensity (Dou et al., 2018). Quantitative nutrient management (QNM) (Hoshi et al., 1999) is a novel nutrient solution (NS) management method where nutrients are regularly and quantitatively added to the nutrient solution regardless of the EC value and nutrient concentration (Terabayashi et al., 2004; Tsukagoshi et al., 2015). Moreover, QNM has been used to control vegetable quality in recent years (Sago et al., 2018; Tsukagoshi et al., 2021), and the nutrient content in NS significantly affect the antioxidant content in vegetables (Chishaki et al., 1997; Kiferle et al., 2013; Jakovljević et al., 2019).

The light spectrum is also an important environmental condition for regulating the quality of vegetables and herbs (Ohashi-Kaneko et al., 2007; Ilić and Fallik 2017). Besides, red and blue light are often combined as light sources for vegetable production in PFAL (Pennisi et al., 2019; Song et al., 2020), and different R:B ratios significantly affect the antioxidant content of vegetables and herbs (Samuolienė et al., 2016; Dou et al., 2017). Besides secondary metabolite accumulation, nitrate content is also an important indicator of vegetable quality. Humans can develop methemoglobinemia by eating vegetables with high nitrate content (Mensinga et al., 2003), and the nitrate content in some vegetables is significantly affected by nutrient supply (Sago et al., 2018) and light spectrum (Nájera and Urrestarazu 2019).

The present study investigated the response of secondary metabolite accumulation and nitrate content in basil to the R:B ratios and applied nutrient amounts. We also determined the optimal combination of the two factors to improve the plant quality of basil grown in PAFL.

4.2. Materials and Methods

4.2.1. Plant material and plant cultivation

Basil (*Ocimum basilicum* L. var. *basilicum* L. cv. Genovese, Takii & Co., Ltd., Kyoto, Japan) seeds were sown in sponge cubes for germination. The sponge cubes were soaked with an NS (EC = 2.0 dS m^{-1} and pH = 6.5) (Otsuka hydroponic composition, OAT Agrio Co., Ltd., Tokyo, Japan), with the same elemental composition of the NS as in the previous study (Sun et al., 2016). The seedlings were placed under light-emitting diode (LED) lamps (photosynthetic photon flux density = 200 µmol m^{-2} s^{-1}) after germinating.

The seedlings were subjected to three different light spectra and three applied nutrient amounts 20 days after germination. The light spectrum treatments were R:B ratios of 3:7, 7:3, and 9:1, respectively (indicated by RB3:7, RB7:3, RB9:1). Table 1 show the fertilization scheme and the 1T applied nutrient amount derived from our previous study. The photoperiod, temperature, relative humidity, and CO_2 concentration were set to 8/16 h (night/day), 21.3°C/24.5°C (night/day), 60%–80%, and 500 ppm. Air pumps were used to supplement oxygen.

Table 4.1. The applied nutrient amount for 1-10 days, 11-15 days and 16-20 days after transplanting.

Days	Treatments[z]	The applied nutrient amount (mg $plant^{-1}$)					
		N	P	K	Ca	Mg	S
1–10	0.5T	15.05	3.76	21.98	5.69	1.82	1.93
	1T	30.10	7.53	43.96	11.38	3.65	3.86
	2T	60.21	15.05	87.92	22.77	7.30	7.71
11–15	0.5T	16.12	3.56	25.69	8.71	2.19	2.49
	1T	32.24	7.12	51.38	17.41	4.39	4.99
	2T	64.48	14.24	102.76	34.82	8.77	9.98
16–20	0.5T	28.86	7.38	40.29	21.43	4.56	4.02
	1T	57.72	14.76	80.58	42.86	9.13	8.04
	2T	115.44	29.51	161.17	85.72	18.25	16.08

[z]For the treatments, 0.5T, 1T and 2T represent the 3 levels of applied nutrient amount.

4.2.2. Measurements

4.2.2.1. The leaf fresh and dry weights, total leaf area, and leaf mass per area (LMA) of basil:

On the day of harvest, the leaf fresh weight of basil was measured. The basil plants were then oven-dried 3 days at 80°C for to measure the dry weight.

A leaf area meter (Li–3000, Li–Cor, Lincoln, NE, USA) measured the total leaf area. LMA was calculated as the leaf dry weight divided by leaf area.

4.2.2.2. TPC and antioxidant capacity in basil leaves:

The TPC and 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity of basil leaves were determined immediately after harvesting following the methods described previously (Ren et al., 2022).

4.2.2.3 Nitrate content in basil leaves:

The nitrate content of basil leaves was determined immediately after harvest, following the methods described previously (Li et al., 2007).

4.2.3. Statistical analysis

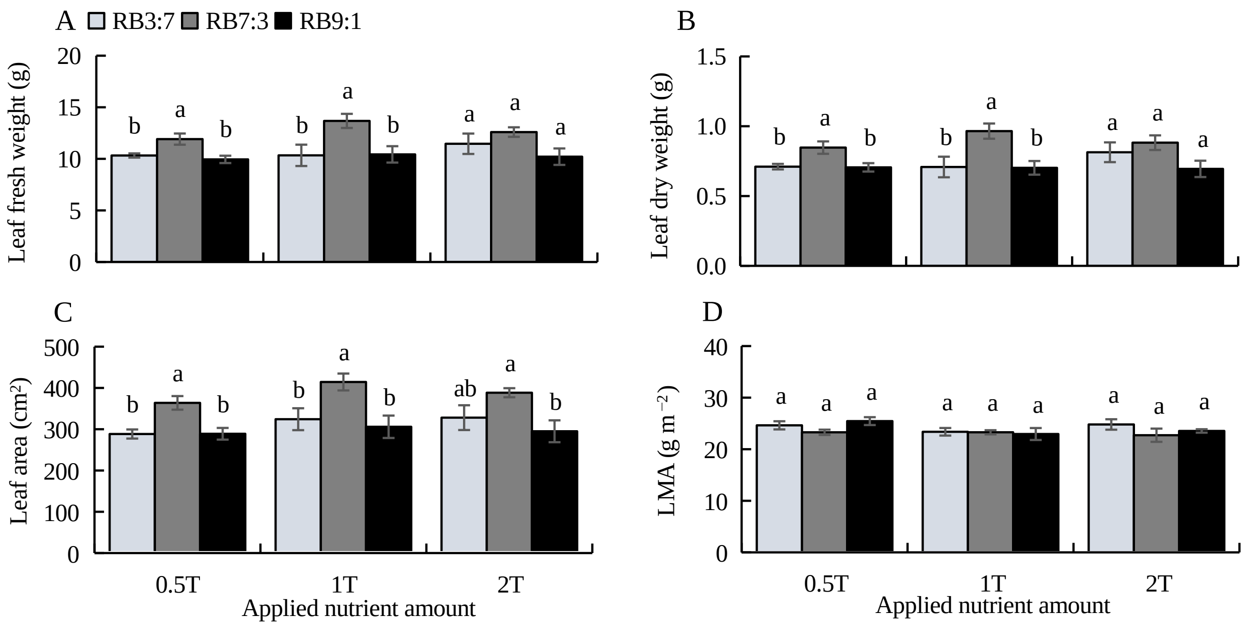
Six replicates were used to test all parameters. The Tukey’s test in SPSS statistical software (IBM SPSS Statistics, Version 19.0. Armonk, NY, USA: IBM Corp.) was used to compare the means of the data. A $P < 0.05$ was used to determine if the difference was significant.

4.3. Results

4.3.1. The response of plant growth to different R:B ratios

Under the applied nutrient amount of 0.5T and 1T, the leaf fresh and dry weights and leaf area were significantly higher at RB7:3 than those at the RB3:7 and RB9:1 (Figures 1A, B, and C). However, different R:B ratios did not affect the leaf fresh and dry weights under the 2T applied nutrient amount (Figures 1A, B) and there was no significant difference of leaf area between the RB3:7 and RB7:3 under the applied nutrient amount of 2T (Figure 1C). Regardless of the applied nutrient amounts, LMA was not affected by different R:B ratios (Figure 1D).

Figure 4.1. Leaf fresh (A) and dry weights (B), leaf area (C) and leaf dry mass per area (LMA) (D) at 20 days after transplanting under different R:B ratios and applied nutrient amount treatments.

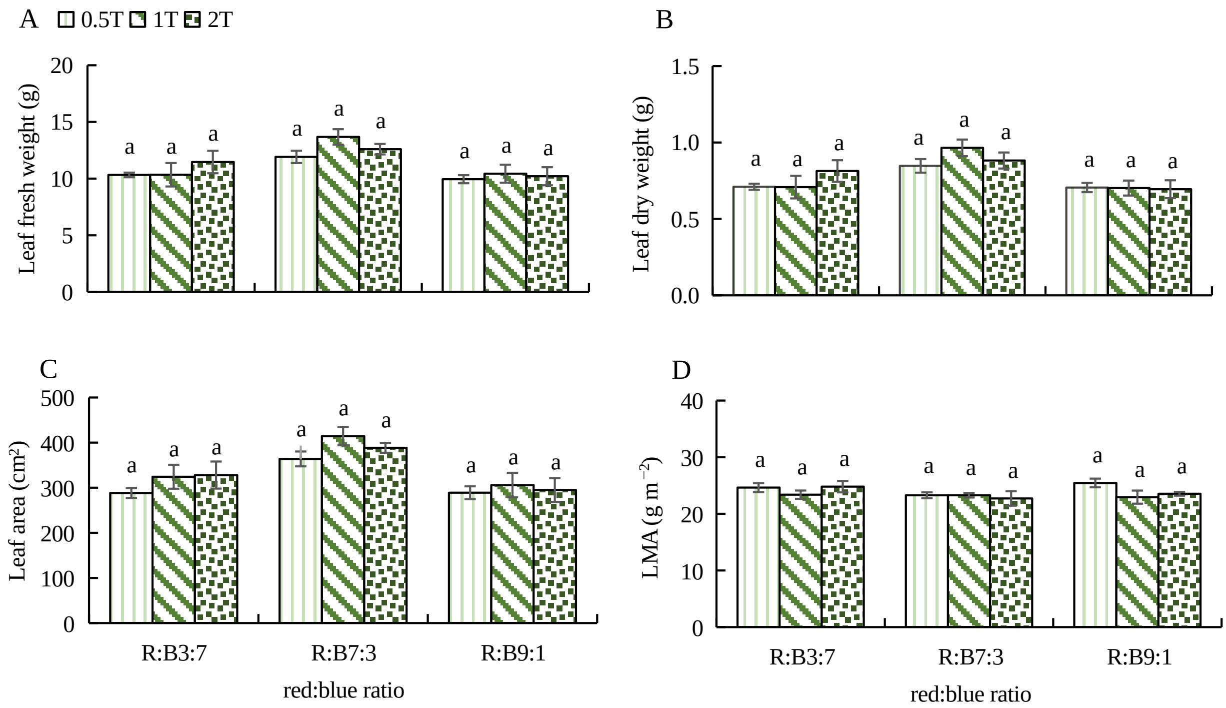


The error bars represent standard error (n = 6). Different lowercase letters indicate significant differences among different R:B ratios based on Tukey's test ($P < 0.05$).

4.3.2. The response of plant growth to different applied nutrient amounts

Different applied nutrient amounts had no effect on the leaf fresh and dry weights, leaf area, and LMA, regardless of R:B ratios (Figures 2A, B, C, and D).

Figure 4.2. Leaf fresh (A) and dry weights (B), leaf area (C) and leaf dry mass per area (LMA) (D) at 20 days after transplanting under different R:B ratios and applied nutrient amount treatments.

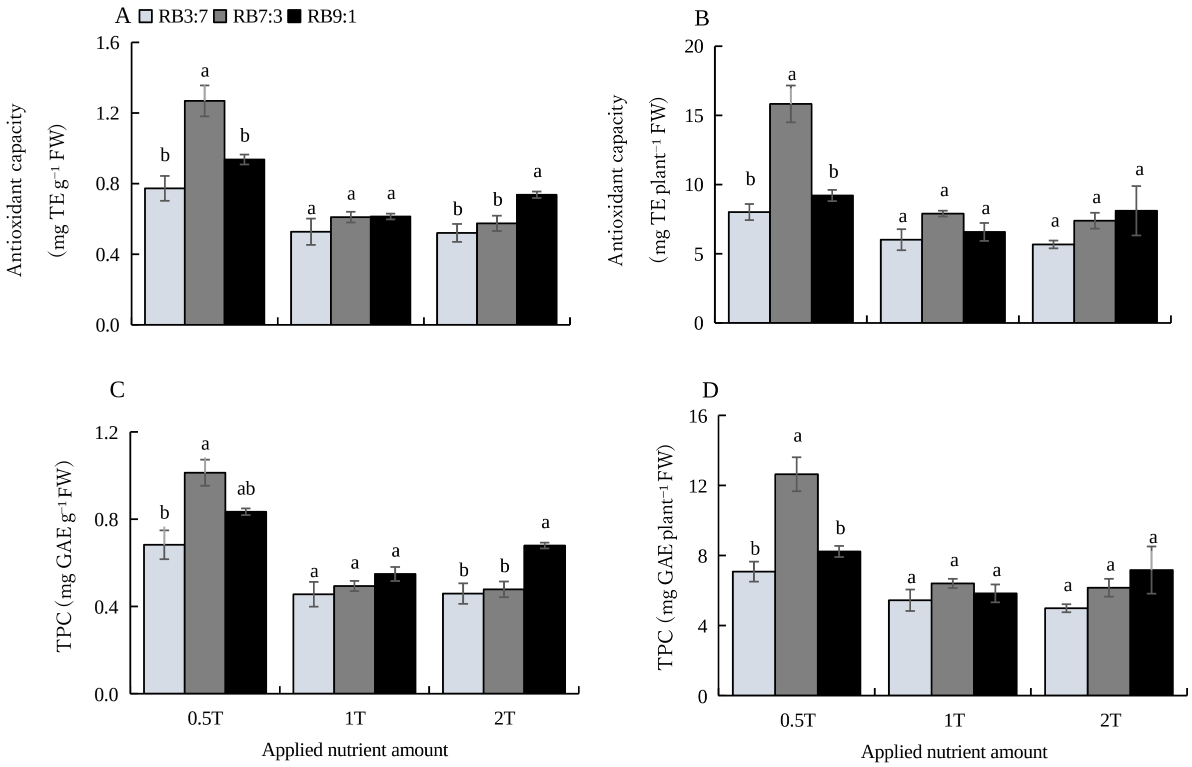


The error bars represent standard error (n = 6). Different lowercase letters indicate significant differences among different applied nutrient amount conditions based on Tukey's test ($P < 0.05$).

4.3.3. The response of antioxidant capacity and TPC to different R:B ratios

Under the 0.5T applied nutrient amount, the antioxidant capacity, antioxidant capacity per plant, and TPC per plant were significantly higher at RB7:3 than those at other R:B ratios (Figures 3A, B, and D). The trend of TPC was similar to the TPC per plant at different R:B ratios and applied nutrient amounts; however, there was no significant difference of TPC between the RB7:3 and RB9:1 (Figure 3C). Under the 1T applied nutrient amount, all the antioxidant capacity, antioxidant capacity per plant, TPC, and TPC per plant were not affected by different R:B ratios (Figures 3A, B, C, and D). Under the 2T applied nutrient amount, the antioxidant capacity and TPC were significantly higher at RB9:1 than those at other R:B ratios; however, there was no significant difference in antioxidant capacity per plant and TPC per plant among different R:B ratios (Figures 3A, B, C, and D).

Figure 4.3. Antioxidant capacity (A), antioxidant capacity per plant (B), TPC (C), and TPC per plant (D) at 20 days after transplanting under different R:B ratios and applied nutrient amount treatments.

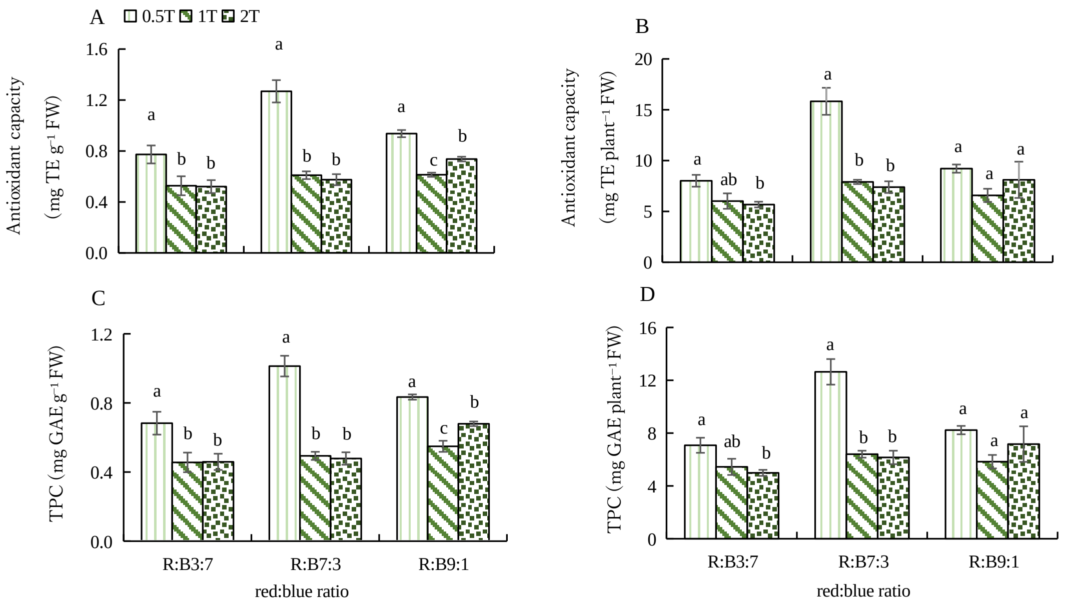


The error bars represent standard error (n = 6). Different lowercase letters indicate significant differences among different R:B ratios based on Tukey's test ($P < 0.05$).

4.3.4. The response of antioxidant capacity and TPC in basil leaves to different applied nutrient amounts

The antioxidant capacity and TPC at the 0.5T applied nutrient amount were significantly higher than those at other applied nutrient amounts regardless of the R:B ratios; however, there was no significant difference of these two parameters between the 1T and 2T applied nutrient amounts under the RB3:7 and RB7:3 (Figures 4A, C). The antioxidant capacity and TPC at the applied nutrient amount of 2T were significantly higher than those at 1T applied nutrient amounts under RB9:1 (Figures 4A, C). The trend of the antioxidant capacity per plant and TPC per plant was similar to the antioxidant capacity and TPC at different R:B ratios and applied nutrient amounts. However, there was no significant difference in the antioxidant capacity per plant and TPC per plant between the 0.5T and 1T applied nutrient amounts under RB3:7. There was no significant difference of the antioxidant capacity per plant and TPC per plant among different applied nutrient amounts under the RB9:1 (Figures 4B, D).

Figure 4.4. Antioxidant capacity (A), antioxidant capacity per plant (B), TPC (C), and TPC per plant (D) at 20 days after transplanting under different R:B ratios and applied nutrient amount treatments.

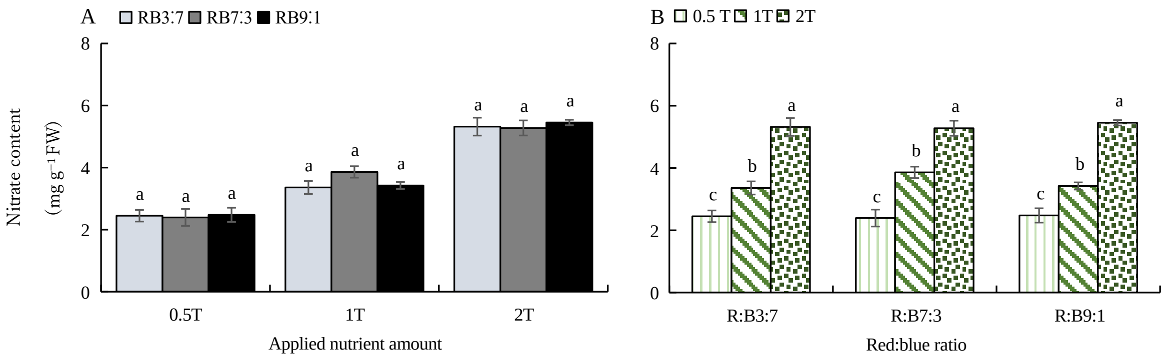


The error bars represent standard error (n = 6). Different lowercase letters indicate significant differences among different applied nutrient amount conditions based on Tukey's test ($P < 0.05$).

4.3.5. The response of nitrate content in basil leaves to different R:B ratios and applied nutrient amounts

Regardless of the applied nutrient amounts, there was no significant difference in the nitrate content in basil leaves among different R:B ratios (Figure 5A). The nitrate content was significantly increased with the increase of the applied nutrient amount, regardless of the R:B ratios (Figure 5B).

Figure 4.5. Nitrate content in basil leaves at 20 days after transplanting under different R:B ratios and applied nutrient amount treatments.



The error bars represent standard error (n = 6). Different lowercase letters indicate significant differences among different R:B ratios (A) and applied nutrient amount conditions (B) based on Tukey's test ($P < 0.05$).

4.4. Discussion

4.4.1. Antioxidant capacity and TPC in basil leaves to different R:B ratios and applied nutrient amounts

In the present study, the antioxidant capacity and TPC were significantly affected by different R:B ratios under the 0.5T and 2T applied nutrient amounts; however, these two parameters were not affected by different R:B ratios under the applied nutrient amount of 1T, indicating that the applied nutrient amount affected the response of secondary metabolite accumulation to different R:B ratios. Moreover, with the increased applied nutrient amount, the increase of red-light ratio promoted the accumulation of antioxidants and TPC (Figures 3A, C). The antioxidant capacity per plant and TPC per plant were significantly higher at the RB7:3 than those at other R:B ratios under the applied nutrient amount of 0.5T (Figures 3B, D), this is because all of the leaf fresh weight, antioxidant capacity, and TPC were promoted by the RB7:3 under the applied nutrient amount of 0.5T.

Compared to other applied nutrient amounts, the antioxidant capacity and TPC were significantly increased by the applied nutrient amount of 0.5T, regardless of the R:B ratios (Figures 4A, C). Moreover, the trend of antioxidant capacity per plant and TPC per plant was similar to the antioxidant capacity and TPC at different R:B ratios and applied nutrient amounts due to the leaf fresh weight was not affected by different applied nutrient amounts. The accumulation of phenolics in plants can be improved by nutrient deficiency. For instance, an NS lacking N, P, and K significantly promoted phenolic substances accumulation in rice (Chishaki and Horiguchi 1997), and an NS with low N and P contents significantly improved the antioxidant content in lettuce (Galieni et al., 2015). Moreover, low concentration NS promoted the accumulation of antioxidants in basil (Jakovljević et al., 2019; Ren et al., 2022) and wrinkled giant hyssop (*Agastache rugosa*) (Lam et al., 2020). The low applied nutrient amount (0.5T) in our study may also induce nutrient deficiency stress on basil, increasing TPC and antioxidant capacity. The improvement of antioxidant capacity in this study may be due to the increase of TPC because total phenolics are the important component of antioxidants in plants (Velioglu et al., 1998; Gonçalves et al., 2013).

4.4.2. Nitrate content to different R:B ratios and applied nutrient amounts

The nitrate content was not affected by different R:B ratios, regardless of the applied nutrient amount. The nitrate content in the NS increases, so does the absorption of nitrate, leading to nitrate accumulation in lettuce (Sago et al., 2018). Our study found that the nitrate content in basil leaves significantly increased with the increase of nitrate content in the NS, and this may also be due to the fact that nitrate uptake increased with the increase of the applied nutrient amount. Similar, the nitrate content increased with the increase of the nitrate content in the NS in lettuce (Fu et al., 2017; Song et al., 2020) and pakchoi (Ding et al., 2018).

4.5. Conclusions

The effects of R:B ratios and applied nutrient amounts on the secondary metabolite accumulation and nitrate content in basil were investigated in this study. Under the 0.5T applied nutrient amount, the RB7:3 is more conducive to the secondary metabolite accumulation than other R:B ratios. Moreover, regardless of the R:B ratios, the antioxidant capacity and TPC were significantly increased at the 0.5T applied nutrient amount as compared to other applied nutrient amounts. Besides, regardless of the R:B ratios, the nitrate content was minimized by the 0.5T applied nutrient amount. Therefore, the combination of RB7:3 and 0.5T applied nutrient amount is optimal for improving the quality of basil plants grown in a plant factory with artificial lighting.

CHAPTER 5. Conclusions and prospects

5.1. Conclusions

Basil (*Ocimum basilicum* L.) has been widely cultivated in PFAL, and for producers, increasing basil yield and quality can increase its economic value. Light quality and nutrient solution management methods have significant effects on basil growth and secondary metabolite accumulation. In addition, since the NS is discharged into the natural environment, it will cause serious waste of resources and environmental pollution. Therefore, improving the yield and quality of basil by optimizing light quality and nutrient solution management methods, and improving the utilization efficiency and absorption efficiency of fertilizers are of great significance for the production of basil.

In chapter 2, the effects of NS with different EC values and different treatment days on the yield, TPC, and antioxidant capacity of basil were studied. The results proved that the yield of basil was optimized with an EC of 3.0 dS m^{-1}; however, the TPC and antioxidant capacity of basil were significantly increased by low ECs of 0.5 and 1.0 dS m^{-1}. Short-term low-EC treatments (0.5 dS m^{-1} for 5 days or water for 3 days) could be used to promote the TPC and antioxidant capacity in leaves without sacrificing yield of basil significantly.

In chapter 3, the effects of different light spectra and applied nutrient amounts in nutrient solution on the growth and fertilizer utilization of basil were studied. The results proved that different applied nutrient amounts did not significantly affect the basil yield, however, the nutrient use efficiency of shoot dry weight was significantly higher at the applied nutrient amount of 0.5T than that at other applied nutrient amounts. Moreover, RB7:3 significantly increased the basil yield under the applied nutrient amounts of 0.5T. Therefore, the combination of the applied nutrient amount of 0.5T and RB7:3 is considered the best for improving the basil yield and nutrient use efficiency in the present study.

In chapter 4, the effects of different light spectra and applied nutrient amounts in nutrient solution on the secondary metabolite accumulation and nitrate content of basil plants were investigated. The results proved that the antioxidant capacity and TPC were significantly improved at the applied nutrient amount of 0.5T. The antioxidant capacity and TPC of basil were significantly increased at RB7:3 under the applied nutrient amount of 0.5T. Moreover, the nitrate content significantly decreased with the decrease of applied nutrient amounts, regardless of the R:B ratios. Therefore, the combination of the applied nutrient amount of 0.5T and RB7:3 is optimal for improving the quality of basil in the present study.

5.2. Future study

Light intensity also affects the growth of basil, the accumulation of secondary metabolites and the utilization efficiency of fertilizers, so the following aspects need further research.

1) The effects of light intensity combined with quantitative fertilization methods on the growth and fertilizer utilization of basil.
2) The effects of photoperiod (continuous light) combined with quantitative fertilization methods on the growth and fertilizer utilization of basil.

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ACKNOWLEDGEMENTS

Thanks for your help in my study and life over the past three years. In particular, Takagaki sensei and Luna sensei, they gave me the opportunity to get a scholarship and allowed me, a person who does not understand Japanese, to live in Japan smoothly for so many years and graduate on schedule. When I came to Japan three years ago, I never expected to face an epidemic that lasted for several years and swept the world, which made it even more difficult for those of us who are studying abroad.

In the past three years, I went from the excitement of getting the news of the scholarship to the confusion of designing the experiment to the difficulties in implementing the experiment to the joy of accepting the paper. Each stage is full of encouragement and help from Takagaki sensei and Luna sensei. I have also been disappointed, but fortunately, with the constant help of Takagaki sensei and Luna sensei, I can get my degree smoothly. Moreover, thanks to Tsukagoshi sensei for teaching me experimental methods and helping me design experiments and revise the paper. Thanks to Xu san and Zhuang san for their help. Thanks to Japanese Government Scholarship Student for my Ph.D. course. In addition to teachers and classmates, I should also thank my parents for their nurturing grace and support for my decisions over the years. Finally, I would like to give a special thanks to my girlfriend (Feng dong xiao) who gave me so much strength and care to get me through the toughest days.