

Research Article

Growth and Carotenoid Contents of Intercropped Vegetables in Building-Integrated Urban Agriculture

Jin-Hee Ju,¹ Sun-Young Cho,² Hee-Yeon Song,¹ Seyoung Ju,² Yong-Han Yoon,¹
and Kyung-Jin Yeum ^{2,3}

¹Department of Green Convergence Technology, College of Science and Technology, Konkuk University, Chungju-si, Republic of Korea

²Department of Integrated Biosciences, College of Biomedical Science, Konkuk University, Chungju-si, Republic of Korea

³Research Institute for Biomedical & Health Science, Konkuk University, Chungju-si, Republic of Korea

Correspondence should be addressed to Kyung-Jin Yeum; kyeum@kku.ac.kr

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Sustainable food security due to climate and social change is more important than ever. This study was conducted to increase plant growth and bioactive contents using intercropping technology in urban agriculture. Tomatoes (*Solanum lycopersicum* L.) and sunflowers (*Helianthus annuus* L.) were intercropped in different ratios in outdoor rooftop, and lettuce (*Lactuca sativa* L.) was grown with chicory (*Cichorium intybus* L.) in the indoor LED plant growth chambers. Carotenoids in plant foods were analyzed using an ultraperformance liquid chromatography with photodiode array detection. Chlorophyll contents were determined by the soil analysis development chlorophyll meter. Tomatoes planted with sunflowers (3:1) had a significantly larger stem diameter ($p < 0.05$), a large number of leaves ($p < 0.05$), and significantly higher lycopene at d88 ($p < 0.05$), d102 ($p < 0.001$), and d115 ($p < 0.01$), and β -carotene contents at d102 ($p < 0.05$) as compared to those of monocultured tomatoes. Lettuce planted in a ratio of 1:3 with lettuce and chicory had significantly higher contents of chlorophyll ($p < 0.05$), β -carotene ($p < 0.05$), and lutein ($p < 0.01$), than lettuce planted alone. On the other hand, intercropping of chicory and lettuce did not have a beneficial effect on the growth and carotenoid content of chicory. The current study indicates that plant growth and carotenoid content can be substantially modified by cocultivation, and the effects may vary depending on the type of plant and the crop ratio.

1. Introduction

Urban agriculture, a food production system of inside city boundaries or densely populated areas, has become an attractive land use alternative in modern society [1]. Urban agriculture's social, economic, and environmental contributions are becoming more important than ever [2]. In particular, there is a new interest in urban agriculture as a means of securing food supply in the context of climate change and other impacts such as the COVID-19 pandemic in all parts of the world [3]. Rooftop gardens, providing an alternative controlled space for growing vegetables, have recently become an important part of urban agriculture revitalization [4, 5]. In addition, indoor farm that can produce plants year-round with controlled lighting is rapidly

expanding in urban and suburban areas [6, 7]. Along with such building-integrated urban agriculture, interest in improving the nutritional and bioactive content of crops is increasing [8–10].

In recent years, growing mix crops has become an important component of sustainable organic farming [11]. Cocultivation is a cross-crop practice often associated with organic farming and can increase in productivity of vegetables per unit area [12]. Growing two or more plants together is usually a method of disease management for crops. However, it can positively change the microclimate conditions around the canopy as well as add organic matter and nitrogen to the soil to retain water and nutrients and control weeds [13]. Additionally, adding noncrop vegetation to a single cultivation helps preserve biodiversity through

resource diversification and reduced use of pesticides [14]. Although these practices can be beneficial to agriculture, they are not practically utilized [15]. Therefore, it is important to demonstrate beneficial effects of easy-to-implement companion planting for urban agricultural farmers and consumers.

Tomato species are a key component of nutrition in many countries and are of great economic importance worldwide [16, 17]. However, continuous single cropping and excessive fertilizer application of tomato plants led to soil acidification and salinization in many areas, reducing tomato yield and fruit quality [18–20]. It has been reported that the yields of tomatoes can be increased when grown with tall sunflower in regions with high light intensities [21]. However, the effect on the bioactive contents in tomatoes grown with sunflowers has not yet been reported.

Lettuce can be a good potential source of bioactive substances such as carotenoids [22], and as Hernandez et al. recently reported [23], their contents can be modified through fertilization management. In addition, LEDs have been reported to improve plant performance and carotenoid content in lettuce [24] and help overcome unpredictable weather conditions [25].

Therefore, we investigated the effects of cocultivation on plant growth and bioactive components by utilizing the outdoor rooftop garden of tomatoes with sunflowers and indoor LED growth chamber of lettuce and chicory in urban agriculture with limited farmland.

2. Materials and Methods

An outdoor roof garden and indoor LED plant growth chamber were utilized to produce plant foods mimicking urban agriculture. Tomatoes (*Solanum lycopersicum* L.) and sunflowers (*Helianthus annuus* L.) were intercropped in different proportions in the roof garden, and lettuce (*Lactuca scariola* L.) was grown with chicory (*Cichorium intybus* L.) in different ratios in LED plant growth chamber as summarized in Figure 1.

The overall conditions of outdoor rooftop gardens and indoor LED growth chamber are presented in Table 1.

2.1. Outdoor Experimental Design: Rooftop Garden. The outdoor experiments were carried out on the rooftop garden of a complex practice building at the university campus (35°49'N, 127°08'N), Chungju-si, South Korea. There were a total of 15 square plots (100 cm length × 100 cm width × 30 cm height) which were made of brick and mortar constructed in full sun at the field experiment rooftop. This plot consists of three layers (from bottom to top): a drainage layer, filter fabric, and growing substrate. The drainage layer had a 25 mm thick drainage board with a high impact polystyrene dimple sheet. A nonwoven geotextile fabric was bonded to the upper surface of the drainage board as the filter layer, which prevented the small particles from being washed from the substrate layer. The growing substrate was filled with a 25 cm depth of commercial substrate (Hansel Green Co., Ltd., Korea) for green roofs

Tomato (*Solanum lycopersicum* L.) and sunflower seedlings (*Helianthus annuus* L.) were obtained from a nursery under natural conditions nearby and transplanted together in the rooftop plots with a plant density of 12 plants per square meter. Experimental treatments were the four-companion planting at different tomato to sunflower ratios as follows: tomato control (T alone, tomato monoculture); tomato : sunflower = 1:1 (T1S1); 2:1 (T2S1); and 3:1 (T3S1). The outdoor experiments on the rooftop garden were conducted using a randomized complete plot design with four treatments and three replications (a total 12 plots) as shown in Figure 2. During the experiments, regular cultural practices were applied as needed, uniformly through all experimental plots, without any additional fertilization during the growing seasons.

Plant height, diameter of the stem, number of leaves, and leaf shape index were recorded for each treatment when plants were at their peak growth time. These parameters have been used to reflect plant growth rates, competitive ability, and resource use [26]. Plant height (cm) was measured with a meter measuring tape at the vertical distance between the upper boundary of the plant and ground level. Stem diameter was determined using Vernier digital calipers (500–153, Mitutoyo Co., Japan) at the plant base.

Tomato fruits were collected three times while fully ripened (early harvest (88 days), midharvest (102 days) and late harvest (115 days)). An average of 30 fully ripe tomatoes from each experimental plot were harvested at each time point and were randomly transferred to the laboratory in plastic bags (Ziploc, Johnson & Son, USA) within 1 h of harvest for carotenoid analysis.

2.2. Indoor Experimental Design: LED Plant Growth Chamber. The indoor experiment was set up in the laboratory using a randomized miniplastic container with nine replicates. Experimental treatments were seven different seed mixture ratios of lettuce seeds (*Lactuca scariola* L., Jeilseed Bio Co., Ltd., Korea) and chicory seeds (*Cichorium intybus* L., Asia Seed Co., Ltd., Koera) as follows: 40 lettuce seeds (L alone), 20 lettuce seeds + 20 chicory seeds (L1C1), 13 lettuce seeds + 27 chicory seeds (L1C2), 10 lettuce seeds + 30 chicory seeds (L1C3), 27 lettuce seeds + 13 chicory seeds (L2C1), 30 lettuce seeds + 10 chicory seeds (L3C1), and 40 chicory seeds (C alone). Companion sowing experiments were conducted in nine replicates for each of the seven seed mixture ratios (a total of 63 miniplastic containers).

Seeds of the seven different mixtures were sown in each miniplastic container (20 oz) containing 15 g perlite (New PerlShine No. 3, Green Fire Chemicals Co., Ltd., Korea) and 65 g of commercial horticultural substrates (Hanpanseung, Samhwa Greenwell Co., Ltd., Korea) from bottom to top. After sowing, miniplastic containers were irrigated with mist water (100 ml), and a thin layer of horticultural substrates is placed over the seed (~5 mm) with each container covered with a plastic cap to maintain moisture content. Seedlings were grown in environmentally controlled LED plant growth chambers (Masuda Co., Ltd., Korea), where three cultivation frames with 150 cm × 70 cm × 35 cm

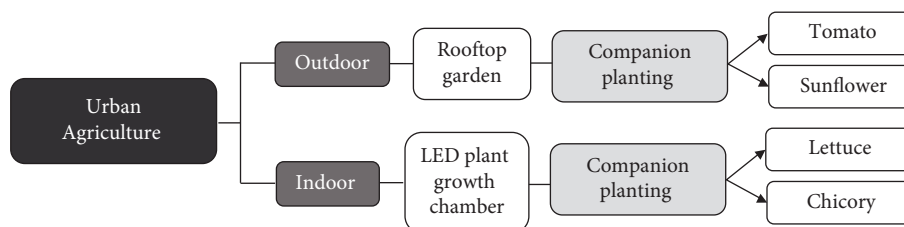


FIGURE 1: Flowchart of the study.

TABLE 1: Growth conditions for companion planting of urban agriculture.

| Condition | Urban agriculture | |
|-------------|---------------------------------------|--|
| | Rooftop garden (tomato and sunflower) | LED plant growth chamber (green lettuce and chicory) |
| Temperature | 23.8°C (11.2~32.1) ^z | 27.7°C (24.7~31.6) |
| Humidity | 66.6% (45.3~93.1) | 37.3% (25.6~53.6) |
| Illuminance | | 10000 lux |

^zMean (minimum~maximum).

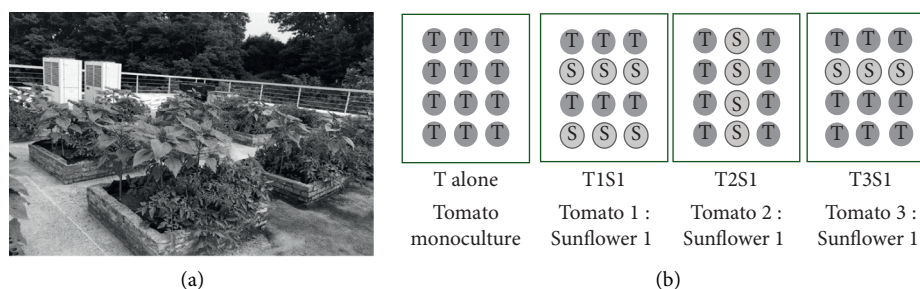


FIGURE 2: The outdoor experimental site (a) and schematic illustration (b) of the companion planting ratios between tomatoes and sunflowers in the plots on the rooftop garden.

(length × width × height) were installed providing the same light treatment as shown in Figure 3.

In the growth chamber, CO₂ concentration was the same as the ambient atmosphere, light intensity was 10,000 lux, the photoperiod was 16 h continuing from 06:00 to 22:00, day/night temperature was 25/21°C, and relative humidity was ~65%. The number of leaves, leaf shape index, chlorophyll contents, and fresh weights were evaluated for nine plants per treatment at day 23 immediately after sowing. The leaf shape index of the third leaf from the basal part was calculated by dividing leaf length by leaf width. Chlorophyll contents were determined by the soil analysis development chlorophyll meter (SPAD-502, Konica Minolta Co., Japan). It was measured in the middle of leaf per each treatment. The fresh weights of shoots and roots were measured using a digital scale (IP65, A&D Co., Japan). At 25 days after sowing (baby leaf stage), lettuce samples were collected in ~60 ml sample bags (LDPE, Cleanwrap Co., Ltd., Korea), wrapped in aluminum foil and immediately transported to the laboratory within 1 h of harvest for carotenoid analysis.

2.3. Carotenoid Analysis. The uniform, nonsenescent, and undamaged edible portion of plant samples were washed with d-H₂O, dewatered, and minced immediately after transport to the laboratory. Samples were extracted for carotenoids and aliquoted 1 g each in a freezer vial and

stored at -80°C for further analysis. All procedures were conducted under red light.

One gram each of plant samples were placed in 50 mL glass vials, and 5 mL of methanol was added and vortexed for 30 sec. Plant samples and methanol mixtures were incubated for 1 hr at room temperature. Afterwards, the mixture was homogenized for 30 s in an ice bath. The mixture was centrifuged at 3000 rpm for 5 min. The methanol layer was transferred into a 50 mL volumetric flask, and the extraction was repeated four times with 10 mL of tetrahydrofuran (THF), followed by vortex and centrifugation. The THF layers were combined with the methanol layer and the volume was brought up to 50 mL. One mL of the extract was taken, dried under nitrogen, and resuspended in 100 μL of ethanol. All extraction procedures for carotenoid analysis were carried out under red light.

Carotenoids were analyzed using a previously reported UPLC-Photodiode Array Detector analysis [27] with minor modification. In brief, the UPLC (ACQUITY UPLC I-Class, Waters Co., Milford, MA, USA) system was equipped with a BEH C18 column (1.7 μm, 2.1 × 50 mm, Waters Co., Milford, MA, USA), binary pump delivery system, autosampler, and photodiode array detector. Mobile phase A was acetonitrile/methanol (7:3, v/v), and mobile phase B was water. Each sample was injected into the BEH C18 column (1.7 μm, 2.1 × 50 mm). The gradient condition was similar to that previously reported [27].

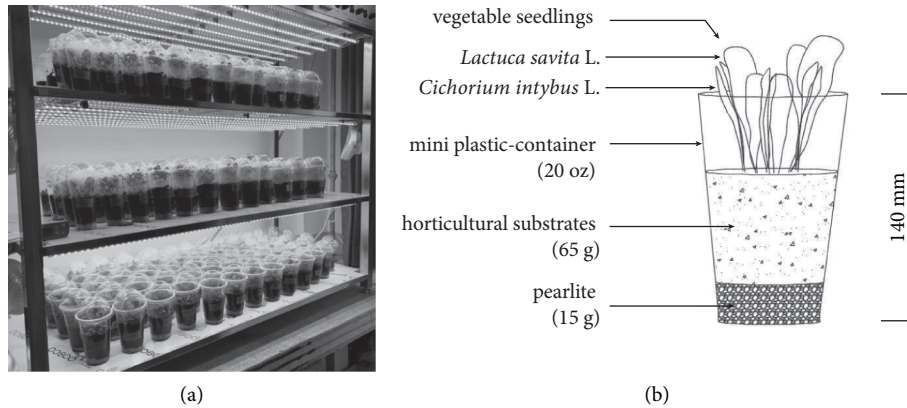


FIGURE 3: The indoor LED plant growth chamber experiments (a) and the cross section of miniplastic container (b) with different ratios of seed mixtures between lettuce and chicory.

β -Carotene, lutein, and lycopene (at 450 nm) were quantified by each standard curve. Each peak was confirmed by retention time and its unique spectrum. The interassay coefficient of variation (CV) was under 4% ($n = 10$), and the intra-assay CV was under 4% as well ($n = 10$).

Representative UPLC profiles of carotenoids from tomato, lettuce, and chicory, and spectra of corresponding carotenoids are shown in Figure 4.

2.4. Statistical Analysis. The experiment was designed as a completely randomized design to test the effect of companion plantings or sowings ratios on all measured parameters. One-way analysis of variance was conducted, and a mean comparison among treatments was conducted using Duncan's multiple range tests ($p < 0.05$). All statistical analyses were performed using SPSS statistical software version 18.0 (SPSS Inc., Chicago, USA).

3. Results and Discussion

The current study reports the companion planting, which was originally used for disease management [28, 29] and improving plant productivity [30], which can be utilized to improve plant growth and bioactive contents in building-integrated urban agriculture. We believe that increasing the availability of a nutritious food supplies through building-integrated urban agriculture can be a useful strategy to overcome food and nutrient instability caused by climate and environmental changes in modern society [31].

3.1. Growth and Carotenoid Contents in Intercropped Tomatoes with Sunflower. In the current study, there was no significant difference in the leaf shape index of tomatoes regardless of the cocultivation rate of tomato and sunflower. However, tomatoes grown in a ratio of 3 to 1 with tomatoes and sunflowers had significantly higher ($p < 0.05$) plant heights, stem diameters, and leaf counts compared to tomatoes grown alone (Table 2).

Considering the height of the sunflower plant was not less than 1.5 m and above the tomato stands 0.5 m, the

tomato plant was protected from direct solar radiation by shady cool air columns. In addition, shading tomato plants creates an ambient microenvironment with high relative humidity, which clearly mitigates moisture loss in tomato plants [32]. On the other hand, the stem diameter and leaf count of tomatoes decreased as the percentage of sunflower cocultivation increased, indicating interspecies competition for sunlight. This suggests that although cocultivation of tomatoes and sunflowers may provide benefits against heat stress as previously reported [33], the increased number of sunflowers may provide tomatoes excessive shading, which can adversely affect their growth parameters.

A green roof with a variety of vegetation structures and colors is a system favored by the public [34]. In the current study, tomatoes and sunflowers were grown together using an outdoor rooftop garden, given that tomatoes are a major component of nutrition in many countries and of economic importance worldwide [35]. White mustard [35] and marigold [28] have also been reported as promising companion crops for tomatoes.

When tomatoes were planted with sunflowers, carotenoid contents in tomatoes were significantly different as compared to those of tomatoes planted alone as shown in Figure 5. Lycopene contents of fully ripened first harvest tomatoes were 5.5 mg and 6.0 mg/100 g FW Edible portion in monoculture and intercropping with lettuce and sunflower, respectively. This lycopene content is similar to the recently reported lycopene contents in cherry tomatoes that abscisic acid, a plant hormone, promoted lycopene accumulation from an undetectable level in green cherry tomatoes to 8 mg per 100 g FW of ripe tomatoes [36]. Companion cultivation of tomatoes with sunflowers at a 3 to 1 ratio yielded 9.6%, 26.9%, and 5.4% higher lycopene contents on days 88, 102, and 115, respectively, than monocultured tomatoes. In addition, the β -carotene content was significantly higher in tomatoes planted in a 3:1 (tomato:sunflower) ratio with sunflowers at 102 days compared to tomatoes grown alone. However, the increase in β -carotene contents by companion planting was not as pronounced as the effect of harvest time. Lutein content was significantly higher in the 1:1 and 3:1 (tomato:sunflower) ratios compared to tomatoes grown alone. On the other hand,

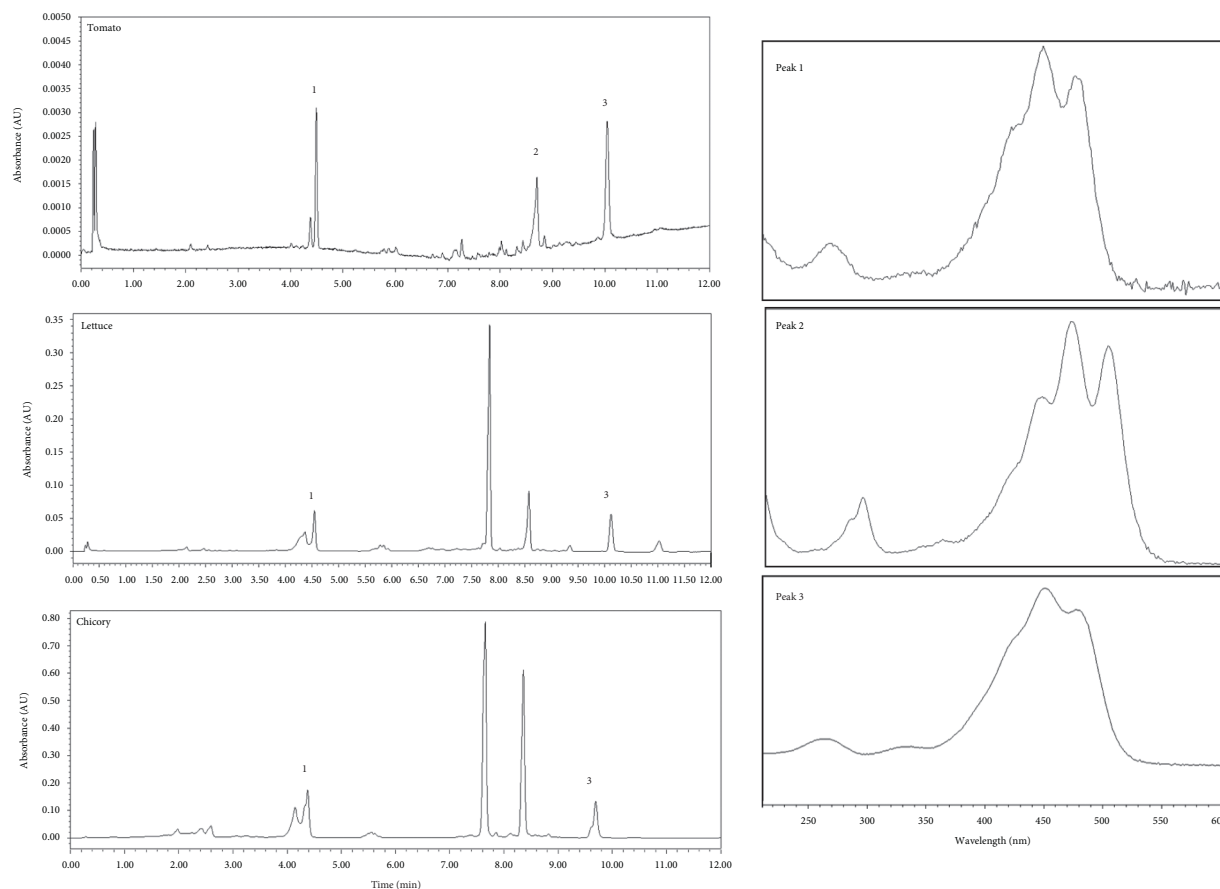


FIGURE 4: Representative UPLC profiles of carotenoids from tomato, lettuce, and chicory, and spectra of corresponding carotenoids.

TABLE 2: Plant growth parameters of tomatoes (*Solanum lycopersicum*) according to companion planting ratio with sunflower (*Helianthus annuus*) in green roofs at 88 days after planting.

| Treatments | Plant height (cm) | Stem diameter (cm) | Number of leaves | Leaf shape index |
|------------|-------------------|--------------------|------------------|------------------|
| T alone | 80.33 ± 0.90c | 17.71 ± 0.38bc | 418.78 ± 9.06b | 1.76 ± 0.03a |
| T1S1 | 105.55 ± 0.83a | 14.75 ± 0.17c | 326.44 ± 2.31c | 1.68 ± 0.02a |
| T2S1 | 86.44 ± 0.68bc | 20.67 ± 0.14ab | 320.89 ± 3.35c | 1.57 ± 0.02a |
| T3S1 | 92.89 ± 0.95b | 23.50 ± 0.71a | 673.33 ± 5.74a | 1.68 ± 0.02a |

T alone, tomatoes; T1S1, tomatoes : sunflower = 1 : 1; T2S1, tomatoes : sunflower = 2 : 1; T3S1, tomatoes : sunflower = 3 : 1. Data are means ± SE. The same letters are not significantly different within the same column according to Duncan's multiple range test at $p < 0.05$ ($n = 9$). Leaf shape index is leaf length/leaf width.

tomatoes grown by companion planting at a 2 : 1 (tomato : sunflower) ratio had significantly lower lutein and α -carotene contents than tomatoes grown alone.

The current study indicates that the carotenoid contents in plant foods can be affected by the intercropping system as well as harvest time. It is interesting to note that the lycopene contents in tomatoes gradually reduced by harvesting time, and β -carotene content increased instead. Considering that the β -carotene contents are ranging from 2.51 mg to 4.77 mg/100 g depending on the harvesting time, and that one cup of tomatoes (200 g) is one serving, one can theoretically obtain 418–795 μ g of retinol by consuming one serving of tomatoes. These results suggest that the harvest time of tomatoes can not only be determined based on the needs of specific carotenoids, but can also be optimized, and that tomatoes can provide significant amounts of carotenoids.

It is well known that lipophilic plant pigments such as carotenoids provide valuable nutritional and health benefits [37]. In addition to provitamin A activity of β -carotene [38], nonprovitamin A carotenoids such as lycopene lower the risk of oxidative stress-associated chronic diseases [39] and lutein has beneficial effects on eye health [40]. Therefore, we believe the results of this study can be the first step in establishing a strategy to achieve food and nutritional security through urban agriculture using intercrop strategies.

3.2. Growth and Carotenoid Contents in Lettuce Intercropped with Chicory. In the LED plant chamber, when lettuce was grown alone or with chicory, there was no significant difference in the number of leaves and fresh weight. However, the leaf shape index of lettuce was significantly lower when

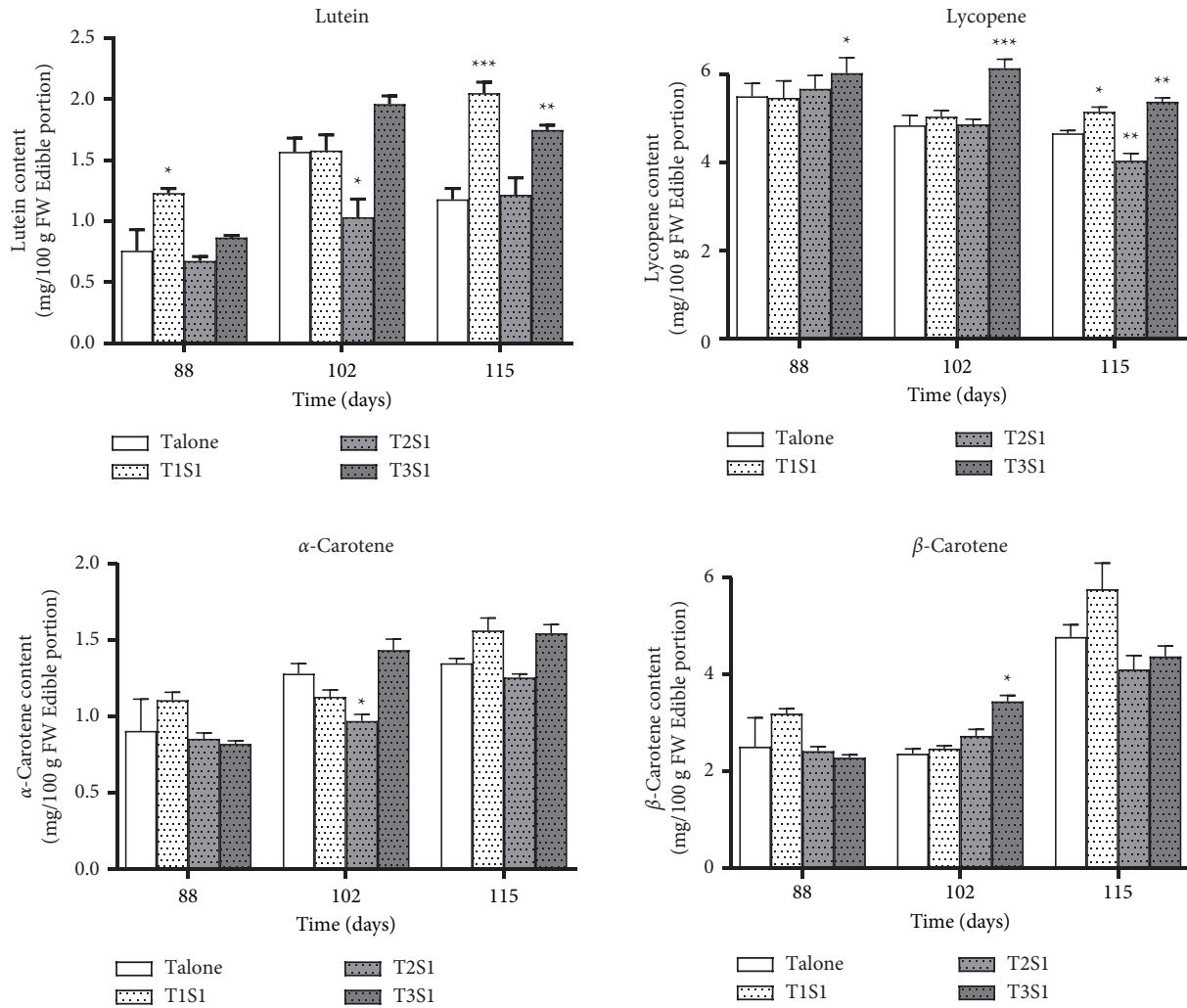


FIGURE 5: Effect of companion planting with sunflowers on carotenoid contents in tomatoes over time grown on the rooftop garden. Carotenoids were analyzed using an UPLC system (means \pm SE, $n = 3$). Significantly different with the monoculture * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

lettuce was cultivated with chicory at a ratio of 1:3 than lettuce grown alone ($p < 0.05$). In addition, the chlorophyll content of lettuce was also significantly ($p < 0.05$) higher in lettuce intercropped with chicory at a ratio of 1 to 3 (Table 3).

It is probable that the companion planting of lettuce and chicory can be beneficial for the strong growth of lettuce as reported earlier [41]. The competition between lettuce and chicory may generate stress conditions, which may lead to the synthesis of secondary metabolites since the general response to (a) biotic stress can be an increase in the synthesis of compounds [42]. On the other hand, chicory intercropped with lettuce had significantly lower leaf shape index, chlorophyll contents, and fresh weight than those planted alone.

The effect of companion planting on carotenoid contents in lettuce are presented in Figure 6.

Lettuce were planted with chicory and harvested 25 days after sowing (baby leaf stage). Carotenoid contents tended to be higher in lettuce grown with chicory. In particular, lettuce grown with chicory at a 1:3 (lettuce:chicory) ratio had

significantly higher β -carotene (6.96 mg/100 g FW edible portion) and lutein contents (6.89 mg/100 g FW edible portion) than lettuce grown alone (β -carotene, 3.66 and lutein, 4.0 mg/100 g FW edible portion, respectively). These carotenoid levels were relatively higher than those reported in Lithuania [43] and were within the range of carotenoid levels of various lettuce previously reported in Korea [44]. It should be noted that the carotenoid content of vegetables can vary depending on a number of factors, including the variety [44], harvest time [45], and extraction procedure [46]. On the other hand, chicory intercropped with lettuce at a ratio of 1 to 1 or 1 to 3 had significantly lower lutein and β -carotene contents than those grown alone as shown in Figure 7.

Lettuce along with chicory represents a fresh leafy vegetable crop with a global production of close to 27 million tons in 2017 [47]. In the cocultivation of lettuce and chicory, the content of β -carotene in lettuce was 15.8–90.1%, and lutein content was 13.5–72.3% higher than that of lettuce grown alone. Considering that β -carotene content of lettuce

TABLE 3: Plant growth parameters of lettuce (*Lactuca savita*) and chicory (*Cichorium intybus*) according to seed mixture ratio in LED plant growth chamber at 25 days after sowing.

| Treatments ^z | Number of leaves ^y | Leaf shape index ^x | Chlorophyll contents (SPAD-value) | Fresh weight (g) |
|-------------------------|-------------------------------|-------------------------------|-----------------------------------|------------------|
| Lettuce | | | | |
| L alone | 7.1 ± 0.38a | 4.1 ± 0.19a | 17.8 ± 1.01bc | 1.50 ± 0.14a |
| L1C1 | 6.7 ± 0.52a | 2.2 ± 0.11c | 17.1 ± 1.55bc | 1.32 ± 0.26a |
| L1C2 | 7.9 ± 0.39a | 2.8 ± 0.15b | 14.8 ± 1.03c | 2.08 ± 0.35a |
| L1C3 | 6.5 ± 0.26a | 2.5 ± 0.10bc | 22.8 ± 0.61a | 2.26 ± 0.56a |
| L2C1 | 7.5 ± 0.33a | 2.4 ± 0.08bc | 20.2 ± 1.28ab | 2.38 ± 0.21a |
| L3C1 | 7.3 ± 0.37a | 3.9 ± 0.25a | 17.9 ± 1.00bc | 1.34 ± 0.23a |
| Chicory | | | | |
| C alone | 5.8 ± 1.13a | 3.2 ± 0.19b | 28.8 ± 2.97a | 0.45 ± 0.17a |
| L1C1 | 4.3 ± 0.47ab | 2.8 ± 0.19b | 26.2 ± 1.04a | 0.11 ± 0.02b |
| L1C2 | 5.5 ± 0.34a | 2.5 ± 0.14b | 25.7 ± 0.90a | 0.23 ± 0.02a |
| L1C3 | 5.1 ± 0.41a | 3.3 ± 0.19b | 25.1 ± 1.70a | 0.20 ± 0.03a |
| L2C1 | 4.6 ± 0.40a | 2.5 ± 0.13b | 25.7 ± 1.00a | 0.11 ± 0.02b |
| L3C1 | 4.7 ± 0.24a | 4.5 ± 0.78a | 21.2 ± 1.37b | 0.14 ± 0.02ab |

Means ± standard error within column followed by the same letter are not significantly different. L alone, lettuce 40 seeds; C1L1, chicory 20 + lettuce 20; C1L2, chicory 13 + lettuce 27; C1L3, chicory 10 + lettuce 30; C2L1, chicory 27 + lettuce 13; C3L1, chicory 30 + lettuce 10; C alone, chicory 40 seeds. Data are means ± SE, the same letters are not significantly different within the same column according to Duncan's multiple range test at $p < 0.05$ ($n = 9$). Leaf shape index is leaf length/leaf width.

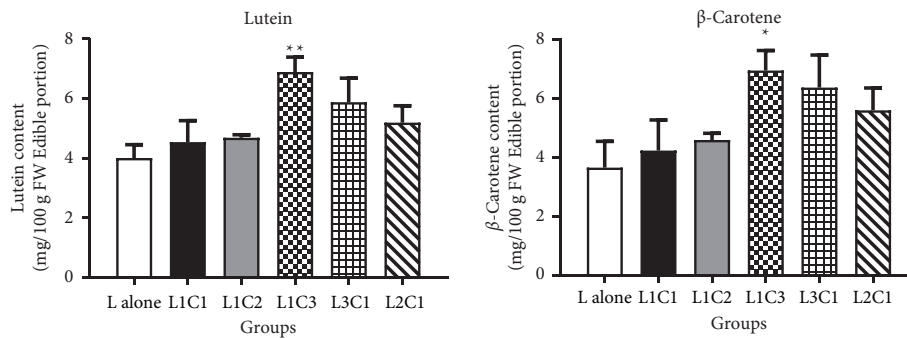


FIGURE 6: Effect of companion planting with chicory on carotenoid contents in lettuce grown in the indoor LED growth chamber for 25 days. Carotenoids were analyzed using an UPLC system (means ± SE, $n = 3$).

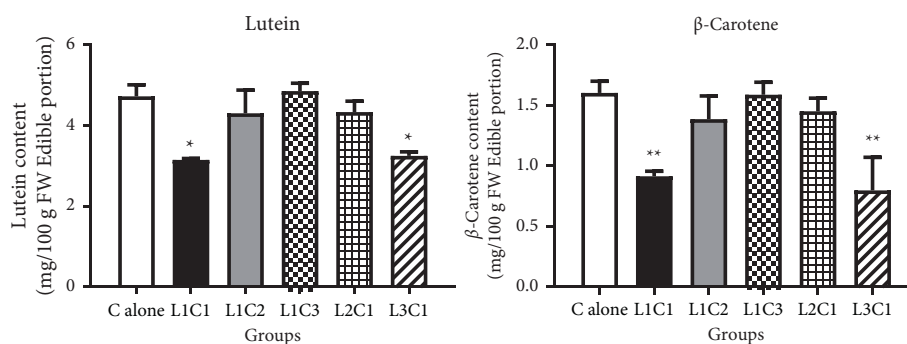


FIGURE 7: Effect of companion planting with lettuce on carotenoid contents in chicory grown in the indoor LED growth chamber for 25 days. Carotenoids were analyzed using an UPLC system (means ± SE, $n = 3$).

can be almost doubled from 3.66 mg/100 g to 6.96 mg/100 g of lettuce by companion planting, the β -carotene intake in one serving of 36 g lettuce can be increased from 1.32 mg to 2.51 mg. It should not be overlooked, however, that the intercropping of lettuce and chicory was not beneficial for chicory in terms of its growth and carotenoid contents.

4. Conclusions

This study evaluated the effect of cocultivation of plant foods widely used around the world using an outdoor rooftop garden and indoor LED plant growth chamber. The substantial modification of plant growth, as well as carotenoid contents by cocultivation, shown in this study suggests that

building-integrated urban agriculture combined with co-cultivation may be a good strategy to improve food and nutritional security.

Data Availability

All the relevant data have been provided in the manuscript. The authors will provide additional details if required.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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