



# Article Low Plant Density Improves Fruit Quality without Affecting Yield of Cucumber in Different Cultivation Periods in Greenhouse

Xiaotao Ding <sup>1,+</sup><sup>(D)</sup>, Wenfeng Nie <sup>2,+</sup><sup>(D)</sup>, Tingting Qian <sup>1,\*</sup><sup>(D)</sup>, Lizhong He <sup>1</sup>, Hongmei Zhang <sup>1</sup>, Haijun Jin <sup>1</sup>, Jiawei Cui <sup>1</sup>, Hong Wang <sup>1</sup>, Qiang Zhou <sup>1</sup><sup>(D)</sup> and Jizhu Yu <sup>1,\*</sup>

- <sup>1</sup> Shanghai Key Lab of Protected Horticultural Technology, Horticultural Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai 201403, China; xiaotao198108@163.com (X.D.); 13851997535@163.com (L.H.); zhanghongmei@saas.sh.cn (H.Z.); jinhaijun@saas.sh.cn (H.J.); cuijiawei@saas.sh.cn (J.C.); wh\_yxl1201@126.com (H.W.); zhou.qiang@dushigreen.com (Q.Z.)
- <sup>2</sup> Department of Horticulture, College of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China; wfnie@yzu.edu.cn
- \* Correspondence: qiantingting@saas.sh.cn (T.Q.); woloveorange2011@163.com (J.Y.)
- + These authors contributed equally to this work.

Abstract: With the development of the economy, the demand for cucumber quality is quickly increasing. The aim of this study was to elucidate the role that plant density plays in leaf photosynthesis, shoot dry matter distribution, yield and quality of cucumber in different cultivation periods under greenhouse conditions. Experimental treatments based on three plant density treatments (2.25, 3.0 and 3.75 plants  $m^{-2}$ ) were conducted in turn during three growth and harvest periods in a year. The results showed that the changes in photosynthesis and weekly yield per unit area were different and dependent on the harvest time, which was mainly induced by temperature and radiation. Interestingly, we found that reducing plant density did not significantly affect the photosynthesis of leaves and did not decrease weekly yield per unit area and total yield. Low-density treatment had the highest weekly yield per plant and total yield per plant in the three harvest periods, the highest ratio of dry matter being allocated to fruits and the highest contents of soluble sugar, total phenols, flavonoid, soluble protein, vitamin C (Vc), chlorophyll and carotenoids in fruits. Moreover, a relatively low nitrite content was found in fruits following low-density treatment. The study indicated that low-density treatment was associated with a high quality of fruits without reducing the cucumber's total annual yield under natural light in the greenhouse. Hence, our study suggests that properly reducing cucumber plant density to 2.25 plants  $m^{-2}$  could be a practicable approach for greenhouses in Shanghai, China.

**Keywords:** photosynthesis; dry matter; biochemical compositions; production; sowing date; semi-closed greenhouse

# 1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the most popular vegetables cultivated in greenhouses worldwide. As reported by FAOSTAT, the total cucumber production (including gherkins) in China (2020) was 72,833.03 million kilos, from 1.28 million cultivated hectares (https://www.fao.org/faostat/en/#data/QCL, accessed on 13 May 2022), accounting for 79.75% of the world's cucumbers. This cucumber yield is mainly produced in simple greenhouses such as plastic tunnels and solar greenhouses. The climate in these greenhouses cannot be better controlled for cultivation and the potential cucumber yield and quality cannot be exhibited. In recent years, a large number of Venlo-type greenhouses have been introduced in China, which improved the greenhouse climate and cucumber yield [1–3].

With the rapid development of the economy and living standards greatly improving, more and more people are considering fruit quality rather than cucumber yield [4–6].



Citation: Ding, X.; Nie, W.; Qian, T.; He, L.; Zhang, H.; Jin, H.; Cui, J.; Wang, H.; Zhou, Q.; Yu, J. Low Plant Density Improves Fruit Quality without Affecting Yield of Cucumber in Different Cultivation Periods in Greenhouse. *Agronomy* **2022**, *12*, 1441. https://doi.org/10.3390/ agronomy12061441

Academic Editors: José Fernando Bienvenido-Barcena, Ming Li and Antonio Bliska Júnior

Received: 18 May 2022 Accepted: 13 June 2022 Published: 16 June 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Cucumbers are low in calories, contain approximately 95% water, 3.6% carbohydrates, 0.65% protein and are a good source of mineral compounds, dietary fiber, vitamin C, vitamin E, retinol equivalent and B-group vitamins, including folic acid, pantothenic acid and phenolic and flavonoid compounds [7,8]. Therefore, the improvements in the quality of cucumbers caused by changing cultivation strategies have been studied in recent years. Adding organic content, such as biochar [9] or arbuscular mycorrhizal fungi [10], to the soil can improve both cucumber yield and fruit quality. Another means of cultivation in greenhouses is to grow cucumbers on mineral wool and irrigate them with inorganic nutrient solution. Although the composition of the nutrient solution remains the same, there are still differences in the quality of cucumbers in different cycles [6], under different  $CO_2$  concentrations [11] and under different solution pHs [12].

Light is the primary driving force of yield. The study result of Marcelis et al. [13] showed that a 1% increase in the light interception of greenhouse vegetables can increase fruit yield by 0.7–1%. Therefore, supplemental lighting has been adopted in cucumber production across the world [14,15]. However, supplemental light is also accompanied by higher energy costs and directly affects the producers' economic benefits. How to make more use of natural light to increase the cucumber yield and improve the fruit quality in greenhouses is still a great challenge. Optimal plant density could also improve the canopy photosynthetic yield by increasing the light penetration to lower leaves in the canopy, which is beneficial for yield and fruit quality [16–18]. Although numerous studies have proven that changing density does have an impact on yield [19,20], there has been little plant density studies focusing on cucumber quality. As proper plant density not only helps to obtain a high yield and increases fruit quality but also saves labor and production materials [21–23], determining the appropriate cucumber density for whole-year growth could provide important economic benefits for growers.

The purpose of the study was to explore the effect of plant density on cucumber yield and fruit quality in different cultivation periods under greenhouse conditions, to improve cucumber fruit quality and to improve economic benefits. In this study, we carried out three density treatments on cucumbers in a Venlo-type greenhouse in Shanghai to study the effects of different seasonal environmental conditions and different plant densities on leaf photosynthesis, dry matter distribution and the yield and fruit quality of cucumbers. From the results of this study, we expected to find an optimal plant density which could improve fruit quality without affecting cucumber yield in Venlo-type greenhouse in Shanghai.

#### 2. Materials and Methods

The experiments were conducted in a semi-closed Venlo-type glass greenhouse at the Chongming base of the National Engineering Research Center of Protected Agriculture (31°34' N, 121°41' E), Shanghai Academy of Agriculture Sciences (Shanghai, China) in 2017–2018. The cucumber variety used in this study was Deltastar RZ F1 hybrid cucumber (Rijk Zwaan Company, De Lier, The Netherlands). The cucumber grew under natural light in a well-heated greenhouse.  $CO_2$  enrichment was carried out by natural ventilation and Air Treatment Unit (ATU) air circulation during cucumber growth. Climate control, irrigation strategy and plant management were performed with reference to Ding et al. [2]. Cucumber plants were irrigated by drip fertigation, which was controlled by computer. The drain nutrient solution was about 20–30% of that applied after transplanting. The original mother nutrient solutions (A and B) were based on Hoagland's solution with modifications and the mother nutrient concentrations are listed in Table 1. The mother nutrient solutions were revised following the advice of Eurofins (branch company of Suzhou, Suzhou, China) by regular nutrient solution testing. EC of 2.0–2.5 dS m<sup>-1</sup> and pH of 5–6 were maintained before cucumber harvest. Once harvesting started, EC of 2.5–3.0 dS m<sup>-1</sup> and pH of 5–6 were maintained.

Outside temperature and radiation sum were automatically monitored by weather station. Measurement of greenhouse temperature was recorded by a climate sensor (Priva, The Netherlands) in greenhouses, which was placed at the top of the canopy and moved up and down based on plant height (about 20 cm higher than plant head). These parameters were automatically recorded at 5 min intervals by computer.

Α	kg/1000 L	Α	g/1000 L	В	kg/1000 L
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	175	$MnSO_4 \cdot H_2O$	180	KNO3	50
EDTA-Fe (13%Fe)	1.5	ZnSO4·7H2O	125	KH <sub>2</sub> PO <sub>4</sub>	25
		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O	280	$K_2SO_4$	10
		CuSO <sub>4</sub> ·5H <sub>2</sub> O	25	MgSO <sub>4</sub> ·7H <sub>2</sub> O	60
		Na2MoO4·2H2O	15		

Table 1. Component elements in the mother nutrient solutions A and B in different tanks.

There were three cultivation periods for cucumbers: (1) the first cultivation period, with sowing date of 8 February 2017, transplanting date of 3 March 2017, end of cultivation date of 2 July 2017, and harvest period of 4 April 2017–2 July 2017(week 14 to 26); (2) the second cultivation period, with sowing date of 16 June 2017, transplanting date of 5 July 2017, end of cultivation date of 7 September 2017, and harvest period of 19 July 2017– 7 September 2017 (week 29 to 36); and (3) the third cultivation period, with sowing date of 26 August 2017, transplanting date of 11 September 2017, end of cultivation date of 2 January 2018, and harvest period of 3 October 2017–2 January 2018 (week 40 to 52, 2017 and week 1, 2018). Cucumber seeds were sown in Grodan blocks (10 cm  $\times$  10 cm  $\times$  6.5 cm) for seedling growth in the seedling greenhouse, in which the climate is well-controlled. In each cultivation period, cucumber seedlings were planted on rockwool slabs (100 cm imes20 cm  $\times$  7.5 cm) in greenhouse and irrigated by drip fertigation. Plants were pruned to a single stem, and one flower was kept at each node (one flower was kept at two nodes at the end of harvest) from the 6th node onwards. When the cucumber yield clearly decreased, we would stop the cucumber growth and harvest. At each end of cultivation, the greenhouse was completely disinfected by 0.3% bleaching powder.

Block design was used in the experiments, with three planting density treatments and four replicate blocks. The three different plant density treatments were designed as follows: (1) low density, with 2.25 plants m<sup>-2</sup>, average distance of plants × row pitch = 55.56 cm × 80 cm; (2) middle density, with 3.0 plants m<sup>-2</sup>, average distance of plants × row pitch = 41.67 cm × 80 cm; (3) high density, with 3.75 plants m<sup>-2</sup>, average distance of plants × row pitch = 33.33 cm × 80 cm. Each density treatment block was set at 48 m<sup>2</sup>. One plant had one stem and used a high-wire growing system (Figure 1).

#### 2.1. Measurements of Cucumber Yield, Dry Weight of Leaves, Petioles, Stem and Fruits

Normally, the cucumbers were harvested every 1–2 days, with the yield recorded each time, and all fruits harvested during the week were added together as the weekly yield. For each harvest period, the first and last week's yield data may be a non-full-week harvest, resulting in a relatively low yield. Normally, 3–5 leaves were pruned each time and 15–18 functional leaves were maintained for plant photosynthesis in each cultivation period. The pruned leaves and petioles were heated in an oven at 105 °C for 2 h and then dried at 80 °C over 3 days to measure their dry weight. At the end of cultivation, the plant leaves, petioles and stems were all collected to dry using the above method and then each sum was calculated. 3–5 plants were selected for each treatment in one block area and repeated the measurement three times. In the harvest period, we collected some fruits together, obtained the fresh fruit weight for each treatment weekly and then dried them completely in an oven and obtained the ratio of fresh weight to dry weight. Referring to the harvest yield, we calculated the total fruit dry weight. During the early fruiting of each harvest period, at least 10 intact fruits obtained under different treatments were selected and crushed into a mixed homogenate to determine the biochemical components of the cucumber fruit.



Low density

Middle density

High density

**Figure 1.** The cultivation arrangement of three density treatments in greenhouses. (**A**), the semiclosed, Venlo-type glass greenhouse used in the experiment. (**B**), picture of the plants, with about 15 leaves unfolding in different density treatments.

### 2.2. Measurements of Outside Temperature, Radiation Sum and Greenhouse Temperature

Measurements of environmental factors were downloaded from the computer for the whole year of 2017. The data for outside temperature, radiation sum and greenhouse temperature were downloaded every half an hour.

## 2.3. Measurements of Leaf Gas Exchange Parameters

A Li-6400 Portable Photosynthesis System (LI–COR, Inc., Lincoln, NE, USA) was used to measure the leaf gas exchange parameters of the net photosynthetic rate ( $P_n$ ). A total of 1000 µmol photons m<sup>-2</sup> s<sup>-1</sup> were established to show the level of irradiance for measurements. The air temperature, CO<sub>2</sub> concentration and relative humidity were those of the greenhouse conditions. The fully developed leaves of different treatments, as above, were acclimated to the irradiance level for approximately 2 min before recording [24]. The

measurements were conducted at approximately 10 a.m. on a sunny day, and measurements were usually taken approximately once every 10 days during the cultivation period.

#### 2.4. Measurements of Fruit Chlorophyll and Carotenoid Contents

The cucumber fruits were collected in a container; the homogenates of fruits were made with a mincer. A total of 0.1 g of fruit tissue was extracted with 95% *v/v* ethanol and measured with a UV–visible spectrophotometer (Ultraviolet–2700; Shimadzu, Tokyo, Japan) at 665, 649 and 470 nm. The chlorophyll (Chl) and carotenoid (Car) contents were calculated as described below:

Chl = chlorophyll a + chlorophyll b =  $((13.95 \times D_{665} - 6.88 \times D_{649}) + (24.96 \times D_{649} - 7.32 \times D_{665})) \times V/(1000 \times W)$ . Car =  $(1000 \times D_{470} - 2.05 \times (13.95 \times D_{665} - 6.88 \times D_{649}) - 114.8 \times (24.96 \times D_{649} - 7.32 \times D_{665})) \times V/(245,000 \times W)$ . V and W represent volume of extracting solution and the sample weight of fruit tissues, respectively.

#### 2.5. Measurement of Soluble Sugar, Soluble Protein, Vitamin C, Total Phenolic and Flavonoid Contents

Soluble sugar content was determined using the anthrone colorimetric method with reference to Dubois et al. [25]. Soluble protein content was measured using Coomassie brilliant blue G–250 staining, with reference to the method of Bradford [26]. Vitamin C was measured using a titration method with 2,6–dichlorophenol [27]. Total phenolic and flavonoid contents were determined by test kit (Suzhou Comin Biotechnology CO., Ltd., Suzhou, China) according to Dewanto et al. [28].

## 2.6. Measurements of Nitrate and Nitrite Contents

The fresh fruit tissues (2 g) received from homogenates by mincer were added to a test tube with 10 mL distilled water. The test tube was heated in boiling water for 30 min and then cooled down with tap water. The extract in the tube was filtered into 100 mL flask, the residue was repeatedly washed and finally distilled water was added to the flask to make a solution of 100 mL. The filtrate was used to determine nitrate and nitrite content. The nitrate concentration of the filtrate was detected using the method of Cataldo et al. [29]. The content of the nitrite in the filtrate was determined by the method of Kaur et al. [30].

#### 2.7. Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted using SAS v. 9.3 (SAS Institute Inc., Cary, NC, USA). Each value was presented as the mean  $\pm$  standard deviation (SD), with a minimum of three replicates. Differences between the treatment means were tested using the Least Significant Difference (LSD) method at  $\alpha$  = 0.05 level of significance. The figures were plotted using Origin 8.5 (OriginLab, Northampton, MA, USA).

#### 3. Results

#### 3.1. Environmental Changes in Greenhouse

The outside temperature showed waveform changes from January to December in 2017 (Figure 2). The minimum outside temperature  $(-2.9 \degree C)$  was measured on 11 February 2017, and the maximum temperature (39.79 °C) was measured on 24 July 2017. The minimum temperature of the greenhouse did not widely vary and looked somewhat linear in 2017 (Figure 2). The highest temperatures in the greenhouse (such as 44.97 °C) occurred on days when windows were almost closed (for short-term heat shock). The daily radiation sum gradually increased from January to mid-June, decreased in late June, remained at a high level from July to September, and a gradual decrease from October to December.

The maximum, minimum and average outside temperatures of the second harvest period (19 July 2017–7 September 2017) were 6.60 °C, 12.99 °C and 8.56 °C higher, respectively, than those of the first harvest period (4 April 2017–2 July 2017), and 10.19 °C, 23.77 °C and 16.79 °C higher, respectively, than those of the third harvest period (3 October 2017–2 January 2018) (Table 2). The maximum, minimum and average greenhouse temperatures of the second harvest period were 1.93 °C, 4.50 °C and 4.21 °C higher, respectively, than

those of the first harvest period, and 5.20 °C, 7.02 °C and 7.19 °C higher, respectively, than those of the third harvest period. The average daily radiation sum of the second harvest period was 0.086 KJ m<sup>-2</sup> higher than that of the first harvest period and 0.876 KJ m<sup>-2</sup> higher than that of the third harvest period. The maximum daily radiation sum of the third harvest period was only 1.833 KJ m<sup>-2</sup>.



**Figure 2.** Changes in greenhouse temperature, daily radiation sum and outside temperature in 2017. Data for greenhouse temperature and outside temperature were recorded every half an hour.

**Table 2.** The maximum, minimum and average outside temperature, greenhouse temperature and radiation sum for different harvest periods. Max, Min, AV represent the maximum, minimum, average values of the climate parameters.

Climate Parameters	The First Harvest Period (4 April 2017–2 July 2017)			The Second Harvest Period (19 July 2017–7 September 2017)			The Third Harvest Period (3 October 2017–2 January 2018)		
·	Max	Min	AV	Max	Min	AV	Max	Min	AV
Outside temperature (°C)	33.19	8.1	20.26	39.79	21.09	28.82	29.6	-2.68	12.03
Greenhouse temperature (°C)	36.27	16.2	23.11	38.2	20.7	27.32	33	13.68	20.12
Daily radiation sum (KJ $cm^{-2}$ )	2.945	0.160	1.696	2.715	0.480	1.782	1.833	0.119	0.906

## 3.2. Cucumber Yield Changes in Three Plant Density Treatments

The cucumber weekly yield per unit area for different treatments showed seasonal variations, with no significant difference in all treatments (Figure 3). There were 13 weeks' harvest time in the first cultivation period. The highest weekly yield appeared in the third and the fourth weeks, gradually decreased from the fifth to the tenth weeks and remained low until the end of the harvest.

The second cultivation period was only 8 harvest weeks. The highest yield appeared at the second week and then gradually decreased until the fifth harvest week. The last harvest time obtained the lowest weekly yield per unit area because the harvest only lasted two days (Figure 3).



**Figure 3.** Changes in weekly yield per unit area and weekly yield per plant under different density treatments in three harvest periods. The data represent the mean  $\pm$  SD (n = 4).

There were 14 harvest weeks in the third cultivation period. The cucumber weekly yield per unit area decreased from the fourth to the twelfth weeks. Yield increased slightly in the thirteenth week, with the lowest output being in the last harvest week (Figure 3).

For different plant density treatments, the changes in cucumber weekly yield per plant were similar to those in the weekly yield per unit area (Figure 3) but the yields per plant for those with low-density treatment were always higher than those for middle- or high-density treatments, and yields per plant for the middle-density treatment were higher than those for the high-density treatment. This difference was particularly evident in the first harvest period.

From the annual total yield per unit area, it can be seen that there was no significant difference in total cucumber yield over the unit area for low-density, middle-density and high-density treatments (Figure 4). That is, changing the density had no significant effect on the yield per unit area. Cucumber total yield per plant over the three harvest periods using the low-density treatment was significantly higher than that for plants with the middle-density treatment, and the yield for the middle-density treatment was significantly higher than that for high density treatment (Figure 4). This indicates that reducing the planting density promoted the yield per plant.



**Figure 4.** Cucumber total yield per unit area and total yield per plant of different density treatments in three harvest periods. The data represent the mean  $\pm$  SD (n = 4). Different letters indicate significant differences at  $\alpha$  = 0.05 based on the Least Significant Difference (LSD) test.

## 3.3. Leaf Photosynthesis and Dry Matter Distribution for Different Plant Density Treatments

Different density treatments had no significant effect on leaf net photosynthetic rate  $(P_n)$ . However, the  $P_n$  significantly changed at different fruiting stages (Figure 5). As the fruiting period progressed, the leaf  $P_n$  gradually decreased. At the end of the harvest, this decrease was more obvious. The  $P_n$  of leaves in spring and autumn could be maintained at a high level for a long time, while the  $P_n$  of leaves in summer rapidly decreased, with a shorter fruiting period.



**Figure 5.** Changes of cucumber net photosynthetic rate ( $P_n$ ) of different density treatments in three cultivation periods. The data represent the mean  $\pm$  SD (n = 6).

The dry matter of the cucumber stems, petioles, leaves and fruits were measured at the end of each cultivation. Figure 6 shows that, across all density treatments and cultivation periods, shoot dry matter was most distributed in fruits, followed by leaves, then stem, and the lowest distribution was found in petioles. There were obvious differences in organ dry matter under different density treatments. The dry matter allocated to fruits under the low-density treatment was 47.57%, while those under middle-density and high-density treatments were measured at 45.03% and 39.43%, respectively. The three pie charts in Figure 6 show that the distribution of dry matter to leaves, petioles and stem increased with the increase in planting density, which illustrates that, with an increase in cultivation density, the cucumber allocates more dry matter for vegetative growth. However, distribution adjustment did not increase dry matter production but resulted in a gradual decrease in the total dry matter weight per plant as the cultivation density increased. The total dry weight per plant over the three cultivation periods was 1088.8 g in the low-density treatment, 896.2 g in the middle-density treatment and only 703.7 g in the high-density treatment.



**Figure 6.** Cucumber plant dry matter distribution of different density treatments in three cultivation periods. The data represent the mean  $\pm$  SD (n = 3). Different letters indicate significant differences at  $\alpha$  = 0.05 based on the Least Significant Difference (LSD) test.

## 3.4. Cucumber Fruit Quality for Different Plant Density Treatments

The measured cucumber fruit quality included soluble sugar, total phenolic, flavonoid, solute protein, Vc, chlorophyll, carotenoid, nitrate and nitrite contents, as shown in Figure 7. In most cases, the soluble sugar, total phenolic, flavonoid, soluble protein, Vc, chlorophyll and carotenoid contents in cucumber fruits under low-density treatment were significantly higher than for fruits of the middle- and high-density treatments in different cultivation

periods, while the nitrite contents showed the reverse results. There were no significant differences in nitrate content among the different treatments over different cultivation periods. The soluble sugar, total phenolic, flavonoid, Vc and chlorophyll contents of cucumber fruit following high-density treatment were the lowest for the three treatments over different cultivation periods. The soluble sugar and Vc contents significantly increased with the decrease in planting density, which indicates that changing the density affects the nutrition and taste of cucumbers. Most of the fruit quality parameters for different density treatments exhibited similar change trends in different cultivation periods.



**Figure 7.** Cucumber fruit quality parameters for different density treatments in three cultivation periods. The data represent the mean  $\pm$  SD (n = 3). Different letters indicate significant differences at  $\alpha$  = 0.05 based on the Least Significant Difference (LSD) test.

#### 4. Discussion

Increasing the yield by concentrating on plant density is a common strategy in cultivation practice [23,31,32]. In this study, we conducted three different plant density treatments to monitor the effect of plant density on the yield of cucumbers as well as to continuously test the variations in fruit yield over the seasons. The results showed that the yield per area for cucumbers showed no significant differences among the three treatments, indicating that cucumber yield per unit area is not a simple linear relationship where a higher plant density of cucumber plants leads to a higher yield of cucumbers. On the contrary, the results of the distribution ratio of dry matter in each organ showed that the low-density treatment had the highest percentage of dry matter being allocated to the fruit, while the lowest percentage of dry matter was allocated to the leaves, petioles and stems (Figure 6), indicating that the low-density treatment is more conducive to the accumulation of dry matter in fruits. The photosynthetic capacity of the adjacent leaves of the fruit directly determines the fruit growth rate [33,34]. Selecting a proper planting density can optimize light interception and increase crop water productivity [35]. The plants growing with a low density easily absorbed the section of light that penetrated into the middle and lower parts of the canopy, resulting in an enhanced photosynthetic rate for leaves related to the fruiting, which was more conducive to the accumulation of fruit dry matter [36].

In this study, we also found an effect of density on the biochemical compositions of cucumbers. The chlorophyll content of the fruits with a density of 2.25 plants  $m^{-2}$ significantly increased, and the color of the peel was significantly darker. Our results indicate that the soluble sugar, total phenolic, flavonoid, soluble protein, Vc and carotenoid contents in fruits are the highest in the fruits grown with a low-plant density. Several studies have shown that the impact of light during fruit ripening appears to be specific to the regulation of pigment accumulation by influencing the transcription of fruit ripening genes or enzymatic activities related to primary or secondary metabolism [37,38]. Cucumber peel has high amounts of chlorophylls, which have a crucial effect on fruit color and taste [39,40]. Glycine, serine and sugar derivatives in the peel determine fruit sweetness [41]. The results of our study confirmed that, in a low-density canopy, the higher the cucumber fruit leaf chlorophyll content, the higher the sugar content and the better the fruit taste (Figure 7). Flavonoids, phenols and Vc are closely related to antioxidants, which can prevent and suppress various diseases and greatly affect consumer preference for cucumbers [42,43]. High dietary intakes of nitrate and nitrite have been implicated in the etiology of human gastric cancer, and approximately 5% of all ingested nitrate is converted in saliva and the gastrointestinal tract to the more toxic nitrite; therefore, people would like a low nitrite content in vegetables [44,45]. In this study, a low nitrite content was found in low-density treatment, which is beneficial for the consumption of cucumber. Chen Omer also confirmed that a proper low plant density reduced the severity of sweet basil downy mildew, which may be also an important means of improving plant yield and quality [46]. Therefore, the cultivation density can be appropriately reduced to synthesize more chlorophyll in cucumber fruit peel and accumulate more metabolic substances, which not only helps to improve the taste of fruit but also meets consumer needs.

Cucumbers in greenhouses are sensitive to light and temperature. A high temperature can affect the differentiation of apical meristems and the leaf initiation rate, thus shortening the growth process [47,48]. The optimum temperature for cucumber growth is 25–30 °C, and the plants are damaged by temperatures above 35 °C [49]. In this study, the outside and greenhouse temperature during the second harvest period was significantly higher than that in the other two harvest periods, which meant that the harvest process in the second harvest period was significantly shorter, causing a severe decrease in yield. Moreover, light in the third harvest period was the main limiting factor for growth, accounting for only about 50% of the other two harvest periods and resulting in an obviously lower weekly yield than the first harvest period (about 40% of average weekly yield compared to the first harvest period), which is also, to some extent, lower than the second harvest period (about 72% of average weekly yield compared to the second harvest period). Although many modern Venlo-type greenhouses have been equipped with air conditioning systems and supplementary lighting systems to cool air temperature in summer and increase lighting in winter, the cost of environmental regulation cannot be ignored. Whether the economic benefits brought by supplementary lighting in winter can cover the energy costs requires further study.

#### 5. Conclusions

This study demonstrated that the yields of greenhouse cucumbers are significantly affected by the environment, and the yields vary over different seasons. Changing plant density has a significant influence on yield per plant but has no influence on total yield per unit area in a natural light greenhouse. Reducing plant density not only promotes the formation of single-plant yield but also increases soluble sugar, total phenolic, flavonoid, soluble protein, Vc, chlorophyll and carotenoid contents in the fruits, which can improve

the fruit quality. Moreover, nitrite content in the fruit is also the lowest in the low-density treatment, which contributes to a healthy diet. Therefore, properly reducing the plant density of cucumber could not only maintain the yield per unit area but also improve the quality of the fruit.

Author Contributions: Conceptualization, X.D., W.N., T.Q. and J.Y.; data curation, X.D., W.N., T.Q., L.H. and Q.Z.; formal analysis, X.D. and T.Q.; funding acquisition, Q.Z. and J.Y.; investigation, X.D., T.Q., L.H., H.Z., H.J., J.C., H.W. and Q.Z.; resources, X.D., W.N., T.Q. and J.Y.; supervision, Q.Z. and J.Y.; writing—original draft, X.D. and W.N.; writing—review and editing, T.Q. and J.Y. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was funded by the National Key Research and Development Program of China (grant no. 2019YFD1001900) and the Shanghai Science and Technology Committee Program (19DZ2281300). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

**Data Availability Statement:** All data used during the study are available from the author Xiaotao Ding by request (e-mail: xiaotao198108@163.com).

**Acknowledgments:** We thank Yuping Jiang for reviewing the manuscript and providing comments on the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### References

- 1. Zhu, W.M.; Chen, Y.Y.; Yang, S.J.; Wan, Y.H. Shanghai Greenhouse Vegetable Production and Agriculture Tour Experience. *Acta Hortic.* **2008**, 797, 449–453.
- 2. Ding, X.T.; Jiang, Y.P.; Hui, D.F.; He, L.Z.; Huang, D.F.; Yu, J.Z.; Zhou, Q. Model Simulation of Cucumber Yield and Microclimate Analysis in a Semi-Closed Greenhouse in China. *HortScience* **2019**, *54*, 547–554. [CrossRef]
- 3. Qian, T.T.; Zheng, X.G.; Yang, J.; Xu, Y.Y.; Wang, Y.; Zhou, Q.; Lu, S.L.; Ding, X.T. Optimal Utilization of Light Energy in Semi-Closed Greenhouse Using Three-Dimensional Cucumber Model. *Sci. Program.* **2020**, *8855063*.
- Gao, L.Y.; Hao, N.; Wu, T.; Cao, J.J. Advances in Understanding and Harnessing the Molecular Regulatory Mechanisms of Vegetable Quality. *Front. Plant Sci.* 2022, 13, 836515. [CrossRef] [PubMed]
- 5. Zhang, J.P.; Feng, S.J.; Yuan, J.; Wang, C.; Lu, T.; Wang, H.S.; Yu, C. The Formation of Fruit Quality in *Cucumis Sativus*, L. *Front*. *Plant Sci.* **2021**, *12*, 729448. [CrossRef] [PubMed]
- Łaźny, R.; Mirgos, M.; Przybył, J.L.; Nowak, J.S.; Kunka, M.; Gajc-Wolska, J.; Kowalczyk, K. Effect of Re-Used Lignite and Mineral Wool Growing Mats on Plant Growth, Yield and Fruit Quality of Cucumber and Physical Parameters of Substrates in Hydroponic Cultivation. Agronomy 2021, 11, 998. [CrossRef]
- Kapusta-Duch, J.; Leszczyńska, T.; Borczak, B. Influence of Packages on Nutritional Quality of Pickled Chilled Stored Cucumbers. Ecol. Chem. Eng. A 2016, 23, 357–371.
- 8. Alsadon, A.; Al-Helal, I.; Ibrahim, A.; Abdel-Ghany, A.; Al-Zaharani, S.; Ashour, T. The Effects of Plastic Greenhouse Covering on Cucumber (*Cucumis Sativus*, L.) Growth. *Ecol. Eng.* **2016**, *87*, 305–312. [CrossRef]
- Ali, A.B.; Elshaikh, N.A.; Hussien, G.; Abdallah, F.E.; Hassan, S. Biochar Addition for Enhanced Cucumber Fruit Quality under Deficit Irrigation. *Biosci. J.* 2020, 36, 1930–1937. [CrossRef]
- Ali, A.; Ghani, M.I.; Ding, H.Y.; Fan, Y.; Cheng, Z.H.; Iqbal, M. Co-Amended Synergistic Interactions between Arbuscular Mycorrhizal Fungi and the Organic Substrate-Induced Cucumber Yield and Fruit Quality Associated with the Regulation of the Am-Fungal Community Structure under Anthropogenic Cultivated Soil. Int. J. Mol. Sci. 2019, 20, 1539. [CrossRef]
- 11. Dong, J.L.; Li, X.; Nazim, G.; Duan, Z.Q. Interactive Effects of Elevated Carbon Dioxide and Nitrogen Availability on Fruit Quality of Cucumber (*Cucumis Sativus*, L.). J. Integr. Agric. 2018, 17, 2438–2446. [CrossRef]
- Preciado-Rangel, P.; Reyes-Pérez, J.J.; Ramírez-Rodríguez, S.C.; Salas-Pérez, L.; Fortis-Hernández, M.; Murillo-Amador, B.; Troyo-Diéguez, E. Foliar Aspersion of Salicylic Acid Improves Phenolic and Flavonoid Compounds, and Also the Fruit Yield in Cucumber (*Cucumis Sativus*, L.). *Plants* 2019, *8*, 44. [CrossRef] [PubMed]
- Marcelis, L.F.M.; Broekhuijsen, A.G.M.; Meinen, E.; Nijs, E.; Raaphorst, M.G.M. Quantification of the Growth Response to Light Quantity of Greenhouse Grown Crops. In Proceedings of the V International Symposium on Artificial Lighting in Horticulture, Lillehammer, Norway, 21–24 June 2005; Volume 711, pp. 97–104.

- Hao, X.; Zheng, J.M.; Little, C.; Khosla, S. LED Inter-Lighting in Year-Round Greenhouse Mini-Cucumber Production. In Proceedings of the VII International Symposium on Light in Horticultural Systems, Wageningen, The Netherlands, 15–18 October 2012; Volume 956, pp. 335–340.
- 15. Singh, D.; Basu, C.; Meinhardt-Wollweber, M.; Roth, B. LEDs for Energy Efficient Greenhouse Lighting. *Renew. Sustain. Energy Rev.* 2015, 49, 139–147. [CrossRef]
- Wang, S.; Jin, N.; Jin, L.; Xiao, X.M.; Hu, L.; Liu, Z.C.; Wu, Y.; Xie, Y.D.; Zhu, W.; Lyu, J.; et al. Response of Tomato Fruit Quality Depends on Period of LED Supplementary Light. *Front. Nutr.* 2022, *9*, 833723. [CrossRef]
- 17. Alsina, I.; Erdberga, I.; Duma, M.; Alksnis, R.; Dubova, L. Changes in Greenhouse Grown Tomatoes Metabolite Content Depending on Supplemental Light Quality. *Front. Nutr.* **2022**, *9*, 830186. [CrossRef] [PubMed]
- Hao, X.; Guo, X.; Chen, X.; Khosla, S. Inter-Lighting in Mini-Cucumbers: Interactions with Overhead Lighting and Plant Density. In Proceedings of the XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC 2014), Brisbane, Australia, 17–22 August 2014; Volume 1107, pp. 291–296.
- 19. Russo, V.M. Plant Density and Nitrogen Fertilizer Rate on Yield and Nutrient Content of Onion Developed from Greenhouse-Grown Transplants. *HortScience* 2008, 43, 1759–1764. [CrossRef]
- Calori, A.H.; Factor, T.L.; Feltran, J.C.; Watanabe, E.Y.; de Moraes, C.C.; Purquerio, L.F.V. Electrical Conductivity of the Nutrient Solution and Plant Density in Aeroponic Production of Seed Potato under Tropical Conditions (Winter/Spring). *Bragantia* 2017, 76, 23–32. [CrossRef]
- Jovicich, E.; Cantliffe, D.J.; Stoffella, P.J. Fruit Yield and Quality of Greenhouse-Grown Bell Pepper as Influenced by Density, Container, and Trellis System. *HortTechnology* 2004, 14, 507–513. [CrossRef]
- 22. Obaid, A.A.; Khalil, N.H.; Al-Alawy, H.H.; Fahmi, A.H. Effect of Planting Density, Foliar Spraying and Overlapping System on the Growth and Productivity Using Soilless Culture System. *J. Saudi Soc. Agric. Sci.* 2022, *in press.*
- Logendra, L.S.; Gianfagna, T.J.; Specca, D.R.; Janes, H.W. Greenhouse Tomato Limited Cluster Production Systems: Crop Management Practices Affect Yield. *HortScience* 2001, *36*, 893–896. [CrossRef] [PubMed]
- Ding, X.T.; Zhang, H.M.; Qian, T.T.; He, L.Z.; Jin, H.J.; Zhou, Q.; Yu, J.Z. Nutrient Concentrations Induced Abiotic Stresses to Sweet Pepper Seedlings in Hydroponic Culture. *Plants* 2022, 11, 1098. [CrossRef]
- 25. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugar and related substance. *Anal. Chem.* **1956**, *3*, 350–356. [CrossRef]
- 26. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
- 27. Qi, Z. Instruction in Plant Physiological Experiment; China Agricultural Press: Beijing, China, 2003.
- Dewanto, V.; Wu, X.Z.; Adom, K.K.; Liu, R.H. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. J. Agric. Food Chem. 2002, 50, 3010–3014. [CrossRef] [PubMed]
- Cataldo, D.A.; Maroon, M.; Schrader, L.E.; Youngs, V.L. Rapid Colorimetric Determination of Nitrate in Plant Tissue by Nitration of Salicylic Acid. *Commun. Soil Sci. Plant Anal.* 1975, 6, 71–80. [CrossRef]
- 30. Kaur, R.; Gupta, A.K.; Taggar, G.K. Nitrate Reductase and Nitrite as Additional Components of Defense System in Pigeonpea (*Cajanus Cajan*, L.) against Helicoverpa Armigera Herbivory. *Pestic. Biochem. Physiol.* **2014**, 115, 39–47. [CrossRef]
- Papadopoulos, A.P.; Ormrod, D.P. Plant Spacing Effects on Yield of the Greenhouse Tomato. Can. J. Plant Sci. 1990, 70, 565–573. [CrossRef]
- Monge-Pérez, J.E. Effect of Pruning and Plant Density on Yield and Quality of Bell Pepper (*Capsicum Annuum*, L.) Grown under Greenhouse Conditions in Costa Rica. *Rev. Tecnol. Marcha* 2016, 29, 125–136. [CrossRef]
- 33. Marcelis, L.F.M. Fruit Growth and Biomass Allocation to the Fruits in Cucumber. 1. Effect of Fruit Load and Temperature. *Sci. Hortic.* **1993**, *54*, 107–121. [CrossRef]
- 34. Marcelis, L.F.M. Fruit Growth and Biomass Allocation to the Fruits in Cucumber. 2. Effect of Irradiance. *Sci. Hortic.* **1993**, *54*, 123–130. [CrossRef]
- Qiu, R.J.; Song, J.J.; Du, T.S.; Kang, S.Z.; Tong, L.; Chen, R.Q.; Wu, L.S. Response of Evapotranspiration and Yield to Planting Density of Solar Greenhouse Grown Tomato in Northwest China. *Agric. Water Manag.* 2013, 130, 44–51. [CrossRef]
- Pao, Y.-C.; Kahlen, K.; Chen, T.-W.; Wiechers, D.; Stützel, H. How Does Structure Matter? Comparison of Canopy Photosynthesis Using One-and Three-Dimensional Light Models: A Case Study Using Greenhouse Cucumber Canopies. *Silico Plants* 2021, 3, diab031. [CrossRef]
- Alba, R.; Cordonnier-Pratt, M.-M.; Pratt, L.H. Fruit-Localized Phytochromes Regulate Lycopene Accumulation Independently of Ethylene Production in Tomato. *Plant Physiol.* 2000, 123, 363–370. [CrossRef]
- Adams-Phillips, L.; Barry, C.; Giovannoni, J. Signal Transduction Systems Regulating Fruit Ripening. Trends Plant Sci. 2004, 9, 331–338. [CrossRef]
- Goularte, A.C.; Capello, C.; Valencia, G.A. Recovery of Chlorophylls from Cucumber (*Cucumis Sativus*, L.) Peel Using a Synthetic Layered Silicate. *Children* 2020, 5, 6.
- Wang, M.; Chen, L.; Liang, Z.J.; He, X.M.; Liu, W.R.; Jiang, B.; Yan, J.Q.; Sun, P.Y.; Cao, Z.Q.; Peng, Q.W.; et al. Metabolome and Transcriptome Analyses Reveal Chlorophyll and Anthocyanin Metabolism Pathway Associated with Cucumber Fruit Skin Color. BMC Plant Biol. 2020, 20, 386. [CrossRef] [PubMed]

- 41. Jo, H.E.; Son, S.Y.; Lee, C.H. Comparison of Metabolome and Functional Properties of Three Korean Cucumber Cultivars. *Front. Plant Sci.* **2022**, *13*, 882120. [CrossRef] [PubMed]
- 42. Masaki, H. Role of Antioxidants in the Skin: Anti-Aging Effects. J. Dermatol. Sci. 2010, 58, 85–90. [CrossRef]
- 43. Fiedor, J.; Burda, K. Potential Role of Carotenoids as Antioxidants in Human Health and Disease. *Nutrients* **2014**, *6*, 466–488. [CrossRef]
- 44. Ding, X.T.; Jiang, Y.P.; Zhao, H.; Guo, D.D.; He, L.Z.; Liu, F.G.; Zhou, Q.; Nandwani, D.; Hui, D.F.; Yu, J.Z. Electrical Conductivity of Nutrient Solution Influenced Photosynthesis, Quality, and Antioxidant Enzyme Activity of Pakchoi (*Brassica Campestris*, L. ssp. Chinensis) in a Hydroponic System. *PLoS ONE* **2018**, *13*, e0202090. [CrossRef]
- 45. Rezaei, M.; Fani, A.; Moini, A.L.; Mirzajani, P.; Malekirad, A.A.; Rafiei, M. Determining Nitrate and Nitrite Content in Beverages, Fruits, Vegetables, and Stews Marketed in Arak, Iran. *Int. Sch. Res. Not.* **2014**, 2014, 439702. [CrossRef] [PubMed]
- 46. Omer, C.; Nisan, Z.; Rav-David, D.; Elad, Y. Effects of Agronomic Practices on the Severity of Sweet Basil Downy Mildew (*Peronospora Belbahrii*). *Plants* **2021**, *10*, 907. [CrossRef] [PubMed]
- 47. Zhu, J.; Andrieu, B.; Vos, J.; van der Werf, W.; Fournier, C.; Evers, J.B. Towards Modelling the Flexible Timing of Shoot Development: Simulation of Maize Organogenesis Based on Coordination within and between Phytomers. *Ann. Bot.* **2014**, *114*, 753–762. [CrossRef]
- Savvides, A.; van Ieperen, W.; Dieleman, J.A.; Marcelis, L.F.M. Phenotypic Plasticity to Altered Apical Bud Temperature in Cucumis Sativus: More Leaves-smaller Leaves and Vice Versa. *Plant. Cell Environ.* 2017, 40, 69–79. [CrossRef]
- Yu, B.W.; Yan, S.W.; Zhou, H.Y.; Dong, R.Y.; Lei, J.J.; Chen, C.M.; Cao, B.H. Overexpression of *CsCaM3* Improves High Temperature Tolerance in Cucumber. *Front. Plant Sci.* 2018, *9*, 797. [CrossRef]