

Article

Effects of Biochar on the Growth and Physiological and Mechanical Properties of Cucumber Plug Seedlings Before and After Transplanting

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Abstract: Since the characteristics of plug seedlings affect the effectiveness of automatic transplanting, this study aimed to explore the effect of the addition of biochar into substrates on the growth of plug seedlings before and after transplanting. The physicochemical properties of substrates with 0%, 5%, 10%, 15%, 20%, and 25% biochar addition all met the requirements of seedling cultivation. The growth trend, root systems, and mechanical properties of seedlings before transplanting and the leaf gas exchange parameters of seedlings after transplanting were measured in this study. The results indicated that the seedlings cultivated with 10% biochar added to the substrate achieved the best growth trend and physiological indices, and the root systems under this treatment were also stronger than those of other treatments, while the seedlings cultivated with 25% biochar treatment were the worst, with less than 22.23% of the growth seen in the 10% biochar treatment, and even less than 1.5% of the growth of the seedlings cultivated without biochar treatment. Since the strong root systems could enhance the mechanical properties of seedling pots, the seedling pots cultivated with 10% biochar added into the substrate possessed the best compression resistance properties, with the maximum value of 49.52 N, and could maintain maximum completeness after free-fall impacting, wherein the loss of root and substrate was only 8.22%. The analysis results of seedlings cultivated after impacting proposed that the seedlings with better growth trends and root systems before transplanting could obtain better leaf gas exchange parameters during the flower stage after transplanting, so the seedlings cultivated with 5%~10% biochar added into the substrate grew better after impacting and then transplanting. It was noticed that the seedlings cultivated with appropriate biochar added into the substrate were able to achieve the optimal growth parameters and mechanical properties before and after transplanting, which were better able to meet the requirements of automatic transplanting. Thus, this study can promote the development of automatic transplanting technology to some extent.

Keywords: plug seedling; biochar; substrate; root system; mechanical property; automatic transplanting



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1. Introduction

As an indispensable and important agricultural product in our daily life, vegetables have become the second largest crop after grains; thus, how to ensure a sufficient supply of vegetables has now become a major livelihood issue [1–3]. As the core aspects of the vegetable industry, the cultivation of plug seedlings and the development of fully automated transplanting technology would effectively increase vegetable yields and bring huge economic benefits [4,5]. In comparison with semi-automatic transplanting and manual transplanting, automatic transplanting has raised higher requirements

for plug seedlings, the reason being that the transplanters are always working at a high speed and that seedlings could easily be damaged if the mechanical properties of the seedling are bad, which would result in a lower seedling transplanting success rate and efficiency [6,7]. Thus, it was important to improve the seedling cultivation technology to obtain seedlings with good mechanical properties that can meet the requirements of high-speed transplanting [8,9].

As the main force-bearing body, the seedling pots, which are mainly composed of substrates and root systems, should have good mechanical properties. Yang proposed that the root systems of seedlings play a key role in wrapping the substrates as a whole, with the root systems being the main subject that suffered significant force during the transplanting process [10]; thus, the seedlings with strong root systems would achieve better mechanical properties. Many scholars have conducted extensive research on the issue of seedling cultivation. Ting added a specific ratio of rock wool with a good fiber structure to the substrate, which could be coiled together with the root system to enhance the strength of the seedling pot [11], and this also indirectly illustrated the importance of the root system for the strength of the seedling pot. Previous research has explored the effects of different substrate ratios and nutrient solution concentrations on the growth quality and root nutrient content of plug seedlings, and the results indicated that the substrate nutrients have a significant impact on the root growth [12], so the nutrients in the substrate could also enhance the seedlings [13]. Different from other methods, the root system growth of cucumber plug seedlings could be significantly promoted by adding a specific ratio of hydrolyzed soy-protein-modified urea formaldehyde resin as an additive to the substrate. Moreover, the modified urea formaldehyde resin could serve as a binder, which could bond the roots and substrate more tightly on the basis of mutual entanglement, and therein the physical and mechanical properties of the seedling pots would be better [14]. The above studies effectively confirmed that the interaction between the root system and substrate could enhance the mechanical properties of plug seedling pots. Therefore, it was important to find a simple and fast seedling cultivation method that could promote seedling growth with the root system.

Biochar is a carbon-rich solid product that is produced by the thermal cracking reaction of agricultural waste such as corn straw under high temperature and limited oxygen conditions [15], and its application has a significant impact on the physical and chemical properties of soil [16]. Regarding the impact on soil physical properties, biochar exhibits a porous structure inside, with a large specific surface area [17], so the rich pore structure of biochar could significantly increase the total porosity and enhance the water-holding capacity of soil [18]. Biochar has a strong cation exchange capacity (CEC), and its application could increase the soil microbial activity and enhance soil stability [19]. Regarding the impact on soil chemical properties, it has a significant improvement effect on soil pH since it is alkaline [20], and it is able to achieve sustained release of NH_4^+ and HPO_4^- ; thus, the effect of improving soil fertility was relatively significant with the improvement of soil fertilizer utilization efficiency [21]. Moreover, the addition of biochar into the soil could significantly increase the soil porosity and reduce the soil bulk density, which could improve the growth environment of crop root systems [22], and the biochar in the soil could reduce the loss of water and nutrients, which could provide sufficient nutrients and water for root systems and crop growth [23].

In light of the advantages of biochar, corn straw biochar was produced by pyrolysis in a tubular reactor, and the effect of the biochar on the growth trend and mechanical properties of plug seedlings before and after transplanting was investigated in this study. Compared with other cultivation methods, the addition of biochar into the substrate could reduce the cost of the substrates, and the seedlings could grow well with an even lower frequency of fertilizer and water application. Moreover, different from other chemical additives, the substrates with biochar addition could improve the structure of the soil instead of affecting the environment after the seedlings were transplanted into the soil. The parameters of the substrates mixed with biochar were measured to ensure that the substrate mixture

could meet the standards for seedling cultivation. By measuring the growth parameters and the mechanical properties of plug seedlings cultivated before transplanting and the leaf gas exchange parameters of seedlings cultivated after transplanting which suffered impacting, the suitable biochar ratio in the substrate for optimal plug seedling cultivation was confirmed, which could meet the requirements of automatic seedling transplanting. Thus, this research provides some basis for the current automatic transplanting technology by optimizing an appropriate vegetable plug seedling cultivation method.

2. Materials and Methods

2.1. Experimental Materials

The experiment was conducted from June to July 2024, under a constant temperature culture room and an experimental sunlight greenhouse with controllable light and temperature conditions, at the Key Laboratory of Modern Agricultural Equipment and Technology, Jiangsu University, Zhenjiang, China (32°20′44″ N, 119°45′27″ E). The average monthly temperatures in June and July were 25.75 °C and 29.5 °C, respectively, during the experimental period, and sunshine was sufficient.

The substrate used to cultivate seedlings in this experiment before transplanting was a mixture of biochar and previous substrate. The corn stover was added to the quartz boat in a tube furnace reactor and heated from room temperature to 550 °C at a heating rate of 55 °C/min, then held at 550 °C for 1 h; the nitrogen was purged for 15 min at a flow rate of 50 mL/min before the reaction so as to obtain an oxygen-free condition throughout the process. Finally, the biochar was obtained when the reaction temperature decreased to room temperature. The previous substrate was mixed with peat (K-413, made by Klasmann–Deilmann Co., Geeste, Germany), perlite, and vermiculite in the proportion of 3:1:1 [24]. Previous research has shown that the growth of seedlings was inhibited when the proportion of biochar in the substrate exceeded 30% [25], so the biochar proportion in the substrate should be below 30%, and we narrowed down the proportion range of biochar to 5% instead of 10% in previous research to obtain more accurate results. Finally, this study used the following ratio of biochar to the previous substrate by weight: 0% (as a control group), 5%, 10%, 15%, 20%, and 25%, which were represented by T₀, T₁, T₂, T₃, T₄, and T₅, respectively.

The seedlings tested in this study were cucumber seedlings (Jinyou1, provided by Cucumber Research Institute in Tianjin Academy of Agricultural Sciences, Tianjin, China). The seedlings were grown in the sunlight greenhouse before transplanting for about 25 days into the 72-hole plastic plug tray (made by Qihang Co., Suqian, China), and each treatment cultivated a whole tray (72 seedlings). Then, the seedlings were chosen to conduct some tests, and the seedlings which had undergone free dropping tests were transplanted into the ordinary plastic flowerpots (21 cm in diameter and 17.5 cm in height, provided by Bailehuan Co., Suqian, China), and cultivated until the end of the flowering period (the total flowering rate was above 95%). The soil which was used after transplanting was loam, and it was selected from Zhenjiang, China; then, it was sieved by passing through a 5 mm mesh before filling the experimental plastic flowerpots. The nutrient solution [26] was poured into the soil every two days in order to ensure the full growth of the seedlings. During the cultivation period, the light exposure time in the greenhouse was beyond 8 h, and the average light intensity was close to 55,000 Lux. The indoor temperature and the air humidity in the greenhouse were maintained at around 30 °C and 60%, respectively, and the greenhouse had good ventilation conditions.

2.2. Physicochemical Properties of the Substrates

For bulk density, the ring knife method was selected for this measurement. When the substrate sample of each ratio was ready, samples were picked by using a ring cutter, and the sample together with the ring cutter was weighed immediately. Meanwhile, the same

samples were filled into the aluminum box for natural air drying, so the unit weight could be described as

$$BD = \frac{m_t - m_c}{V \cdot (1 + \varphi)} \quad (1)$$

where BD is the bulk density, g/cm^3 ; m_t is the total weight of the ring cutter and the sample, g ; m_c is the weight of the ring cutter, g ; V is the volume of the ring cutter, cm^3 ; and φ is the mass water content of the sample, g/kg .

Porosity included the ventilation porosity, water-holding porosity, and total porosity. Firstly, the samples were soaked in the water for 24 h, then the samples were put on the gauze and the water allowed to drip naturally for 24 h, the sample was moved from the gauze and weighed immediately, and finally the samples were put into the oven to dry to stable weight at 105°C ; thus, the porosity could be described as

$$\left\{ \begin{array}{l} P_v = \frac{M_1 - M_2}{V} \times 100\% \\ P_w = \frac{M_2 - M_3}{V} \times 100\% \\ P_t = P_v + P_w \\ V_r = \frac{P_v}{P_w} \end{array} \right. \quad (2)$$

where P_v is the ventilation porosity; P_w is the water-holding porosity; P_t is the total porosity; V_r is the void ratio; M_1 is the weight of the sample which was soaked for 24 h, g ; M_2 is the weight of the sample which dripped naturally for 24 h, g ; M_3 is the weight of the sample which was baked into the stable weight, g ; and V is the volume of the sample, g/cm^3 .

For the pH and EC value, the test samples with distilled water were mixed in a ratio of 1:5, then the mixture was put on the oscillator and vibrated for 30 min, and finally the mixture supernatant was selected for pH and EC value measurement by using AZ-8601 (made by AZ Instrument Co., Taizhong, China) and AZ-8303 (RS232) (made by AZ Instrument Co., Taizhong, China), respectively. It was necessary to ensure that the room temperature was at 26.5°C during the experiment.

2.3. Growth Parameters of the Seedlings

The basic growth parameters were as follows.

- (1) Plant height: the length from the connection point between root and stem to the highest growth point of the seedling, which was measured by ruler (0.1 mm).
- (2) Leaf expansion: the distance between the tips of two main leaves, which was measured by ruler (0.1 mm).
- (3) Stem diameter: the average diameter of the top, middle, and bottom position on the stem, which was measured by an electronic digital vernier caliper (0.01 mm).
- (4) Leaf and stem dry matter: the leaf and stem of the seedlings were put into the oven set at 105°C for 15 min, then the temperature was reset to 80°C to keep drying until the weight was stable, and finally the weight of the leaf and stem dry matters were measured by a precision electronic scale (0.0001 g).

In order to ensure the accuracy of the experimental results, total 72 seedlings in one plug tray were selected for measurement from each treatment and the average value of each treatment was taken for further analysis.

The physiological indices were as follows:

- (1) Regarding the soluble sugar and sucrose of stem and leaf, the sugar content was closely related to crop growth. As shown in Supplementary Figure S1a, 0.1~0.2 g fresh stem and leaf of cucumber plug seedlings was selected, respectively, as samples, then these samples were grounded and crushed, into which a certain amount of prepared extraction solution was added at room temperature. The mixture was then put into a

centrifuge tube with reagent immediately. Finally, the supernatant was extracted and tested by a spectrophotometer.

The soluble sugar content was determined by the anthrone colorimetric method, and the result could be calculated as

$$SSC = \frac{D_a - B_a}{S_a - B_a} \times SS \div \frac{W_F}{V} \times D_r \quad (3)$$

where SSC is the soluble sugar content in the sample, $\mu\text{g/g}$; D_a is the absorbance of the sample solution; S_a is the absorbance of the standard solution; B_a is the absorbance of the blank solution; SS is the concentration of the standard sample, $\mu\text{g/mL}$; W_F is the fresh weight of the sample, g; V is the volume of distilled water for homogenization, mL; D_r is the dilute multiple before sample measurement.

The sucrose content was determined by the resorcinol method, and the result could be calculated as

$$SC = (C_s \times V_1) \times \frac{A_3 - A_1}{A_2 - A_1} \div \left(W \times \frac{V_1}{V_2} \right) \quad (4)$$

where SC is the sucrose content in the sample, mg/g ; C_s is the concentration of the standard tube solution, mg/mL ; A_3 is the absorbance of the sample solution; A_2 is the absorbance of the standard solution; A_1 is the absorbance of the blank solution; W is the weight of the sample, g; V_1 is the volume of the added sample, mL; V_2 is the volume of the added extraction solution, mL.

(2) For the N content of the leaf, as shown in Supplementary Figure S1b, the hyperspectral image acquisition system was designed by our research team. When the two main leaves and one heart leaf grew in cucumber seedling, the heart leaves of each treatment were selected to measure the nitrogen content. In order to reduce measurement errors, six heart leaves were selected for measurement from each treatment and each leaf was measured twice. Previous research has shown that we should measure the edge tip of the leaf (the red shaded area in Supplementary Figure S1b) to ensure the accuracy of the N content [27]. By the way, a certain amount of N contents of leaves was measured by UDK 159 automatic Kjeldahl azotometer (made by VELP Co., Milan, Italy) in order to establish a predictive model.

2.4. Root System Growth Parameters of the Seedlings

The basic growth parameters included the total length, average diameter, and total root tips of the root system. The root system of the seedling was cleaned and organized by using protective cleaning methods [28], and the Perfect V700 root scanner (made by Seiko Epson Co., Suwa City, Japan) was selected to scan the cleaned roots; then, the root analysis software WinRHIZO V2.0 (Professional Edition) was used to process the scanned root data (Supplementary Figure S2).

Regarding the lignin, cellulose, and hemicellulose content of root systems, these major chemical components to some extent reflect the degree of development of the root cell tissues and thus affect the mechanical properties of root system. The cleaned root systems under different treatments were bagged and put into the oven which was set at 75°C to dry until reaching a constant weight; then, the cellulose and lignin content was determined by the iodometric method (acidic detergent) and hemicellulose content was determined by the DNS method (hydrochloric acid hydrolysis) after the samples were crushed and sieved.

Regarding the tensile and shear resistance properties of the root systems, as shown in Supplementary Figure S3, the two properties were mainly tested by the TA.XT plus texture analyzer (made by Stable Micro System Co., Godalming, UK). The cleaned root systems under different treatments were divided into many independent roots, and the tensile and shear resistance properties of each treatment were calculated as the average sum of all independent roots from the same treatment. Since some thin roots were easily broken at the clamped position during tensile testing (Supplementary Figure S3a), the layer of medical rubber adhesive was applied to the two clamped positions (segment ab and segment de

in Supplementary Figure S3a) to improve the successful rate, and it was considered as a successful test if the root fracture point was located in the middle of segment bc in Supplementary Figure S3a, so the tensile strength (Supplementary Figure S3b) could be calculated as

$$P_t = \frac{4F_t}{\pi D_t^2} \quad (5)$$

where P_t is the tensile strength, MPa; F_t is the tensile resistance when the root is broken, N; and D_t is the diameter at the broken site, mm.

And the shear strength (Supplementary Figure S3c) could be calculated as

$$P_s = \frac{F_s/2}{\pi D_s^2/4} = \frac{2F_s}{\pi D_s^2} \quad (6)$$

where P_s is the shear strength, MPa; F_s is the shear resistance when the root is cut off, N; and D_s is the diameter at the broken site, mm.

2.5. Mechanical Property of the Seedling Pots

Regarding the compression resistance property, as shown in Supplementary Figure S3d, the TA.XTplus 100 texture analyzer was also used to conduct this test. Since the shape of the seedling pot was similar to the trapezoidal cone shape, a load-bearing platform was customized based on the shape of the seedling pot to ensure that the seedling pot was in a horizontal state during the compression test, and each treatment was repeated 6 times to verify the accuracy of the test.

Regarding the impact resistance property, the seedlings of each treatment were picked from the plug tray and weighed, then dropped naturally from the height of 0.5 m to the floor, and the seedling was weighed again after impacting, and each treatment was repeated 10 times to verify the accuracy of the test, so the impact resistance property could be calculated as

$$IP = \frac{M_i}{M_c} \times 100\% \quad (7)$$

where IP is the impact resistance strength; M_i is the weight of broken seedling after impacting, g; and M_c is the weight of the complete seedling before falling, g.

2.6. Cultivation of Seedlings After Impacting

The seedlings after impacting for each treatment were cultivated until the flowering stage, and each treatment was repeated 6 times. Since the leaf gas exchange parameters of seedlings (including the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate of the seedling leaves) were an important parameter for measuring seedling physiological indicators, and also an important reflection of seedling photosynthesis, which could bring about 90% of seedling biomass as the main physiological mechanism required for seedling growth [29]. The LI-6400 portable photosynthesis measuring instrument (made by LI-COR Co., Superior Street Lincoln, NE, USA) was used to process this test, and the measurement must be conducted between 9.00 a.m. and 11.00 a.m. with sufficient lighting to ensure the accuracy of the test.

2.7. Statistical Analyses

All experimental data were analyzed by using Excel 2021 (made by Microsoft Co., Redmond, Washington, DC, USA) and SPSS 23.0 (made by IBM Co., Armonk, NY, USA), and all data graphs were made by Origin 2018 (made by OriginLab Co., Northampton, MA, USA). The least significant difference (LSD) method was used for multiple comparisons of these data, and the significance of value (significant when $p < 0.05$) was indicated by different letters.

3. Results and Discussion

3.1. Effect of Physicochemical Properties of Mixed Substrates

The physicochemical properties of substrates on different treatments are shown in Table 1. The results indicated that the bulk density of the substrate significantly decreased as the amount of biochar increased, but other parameters of the substrate showed an upward trend with the increase in biochar content.

Table 1. The physicochemical properties of substrates based on different treatment.

Treatments	Bulk Density/ (g·cm ⁻³)	Ventilation Porosity/%	Water-Holding Porosity/%	Total Porosity/%	Void Ratio	pH Value	EC/ (mS·cm ⁻¹)
T ₀	0.237 a ¹	18.57 b	45.56 d	64.13 c	0.408 a	6.05 b	0.641 c
T ₁	0.224 b	19.47 ab	48.77 c	68.24 bc	0.3998 a	6.15 b	0.73 c
T ₂	0.211 c	20.37 ab	51.98 b	72.35 b	0.392 a	6.26 b	0.82 bc
T ₃	0.206 cd	21.3 ab	52.72 ab	74.02 ab	0.404 a	6.34 ab	0.914 b
T ₄	0.2 d	22.24 a	53.46 a	75.7 ab	0.416 a	6.42 ab	1.009 ab
T ₅	0.194 d	22.75 a	54.1 a	76.85 a	0.421 a	6.58 a	1.08 a

¹ The data in this table were calculated as the average value of different substrate treatments, and the value was significant when $p < 0.05$ by the least significant difference (LSD) multiple comparison method as indicated by different letters in the same column.

Since the biochar has a large specific surface area and porosity [17], the addition of a specific ratio of biochar into the substrate could significantly decrease the bulk density and increase the porosity. However, a substrate which is suitable for crop growth should have appropriate ventilation porosity and bulk density, which is a critical factor to meet the air exchange efficiency during the crop growth process. Similarly, a reasonable water-holding porosity can ensure necessary water for crop growth [18]. In general, the suitable bulk density of the substrates for the growth of plug seedlings was 0.1~0.8 g/cm³, with a total porosity of 54~96%, and the suitable pH and EC values of the substrates for the growth of plug seedlings were 5.5~6.8 and 0.5~2.75 mS/cm [30], respectively. Thus, the physicochemical properties of substrates with the T₀~T₅ treatments were suitable for seedling cultivation.

3.2. Analysis of Seedling Growth Under Different Treatments

3.2.1. Basic Growth Parameters

As shown in Figure 1, the basic growth parameters of seedlings under different treatments all presented a first increasing and then decreasing trend, and all of the parameters were the best when the biochar ratio in the substrate reached 10% (T₂).

The plant height, leaf expansion, and stem diameter of the seedlings under the T₂ treatment increased by 18.35%, 14.24%, and 11.55%, respectively, compared with those under the T₀ treatment. As an important indicator of seedling growth, the leaf and stem dry matter of the seedlings under the T₂ treatment was much higher than that under the T₀ treatment, which reached 24.54%. These basic parameters of the seedlings under the T₁ and T₃ treatments were almost identical, but much higher than that under the T₀ treatment (basically more than 7% higher). However, these parameters presented a decreased trend when the addition of biochar into the substrate exceeded 15% (T₃), and even a significant decreased trend compared with the T₂ treatment when the biochar ratio exceeded 20% (T₄). Furthermore, the parameters of seedlings under the T₅ treatment were surprisingly lower than the substrate without biochar addition (T₀) (basically 1.5% lower).

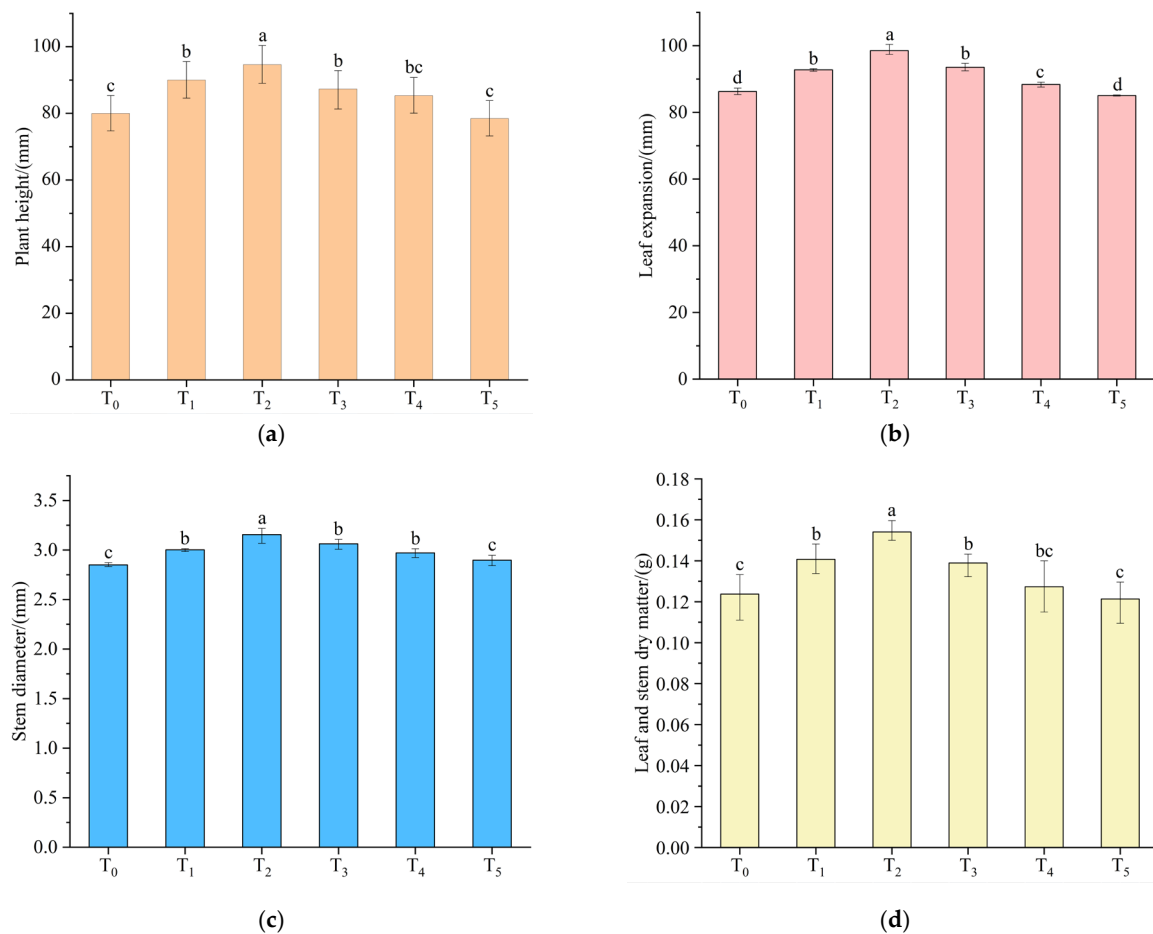


Figure 1. The basic growth parameters of seedlings with different treatments: (a) Plant height; (b) Leaf expansion; (c) Stem diameter; (d) Leaf and stem dry matter. The values shown were significant when $p < 0.05$ by the least significant difference (LSD) multiple comparison method as indicated by different letters.

To sum up, the addition of 10% biochar into the substrate provided a significant promoting effect on the growth of the plug seedling, but the biochar ratio in the substrate was not defined as being the more the better. The appropriate proportion of biochar in the substrate could significantly improve its physical and chemical properties such as bulk density and porosity, which provided a favorable growth environment for plug seedlings, but excessive biochar would inhibit the growth of plug seedlings, with results even lower than the substrate without biochar addition.

3.2.2. Physiological Indices

1. Soluble sugar and sucrose of stem and leaf

As shown in Figure 2, the content of soluble sugar and sucrose in plug seedlings showed a first increasing and then decreasing trend as the biochar ratio in the substrate increased, and the soluble sugar content in the leaf and stem of plug seedlings was significantly higher than that of sucrose.

In addition, the sugar content in the leaf of the plug seedlings was significantly higher than that in the stem, and the reason was that the stems of plug seedlings during the seedling cultivation stage were relatively thin and weak while the leaf grew more vigorously, especially for plug seedlings in the suitable transplanting period (25 days old), which would result in significant differences in the sugar content of different parts in seedlings. Taking soluble sugar content as an example, the seedlings cultivated under the T₂ treatment had the highest content in the leaf and stem (18.78% higher than those

under the T_0 treatment), while the T_5 treatment had the lowest content, so the addition of 10% biochar into the substrate could significantly increase the soluble sugar content. Since the higher sugar content could allow crops to achieve greater metabolism, the seedlings cultivated under 10% biochar treatment could obtain stronger vitality and vigorousness [31]. The soluble sugar content of seedlings cultivated under the T_1 treatment was almost similar to that under the T_3 treatment and much higher than that under the T_0 treatment (15.58% and 12.16% higher than those under the T_0 treatment, respectively), while the soluble sugar content of seedlings cultivated under the T_4 and T_5 treatments was much lower than those under the other treatments, even lower than that under the T_0 treatment, where the cell permeability of these seedlings decreased, and the resistance of these seedlings greatly decreased too [31], which led to the poor growth trend of these seedlings. Thus, the addition of biochar into the substrate was still beneficial for seedling growth, but the biochar ratio was not defined as being the more the better, which also confirmed the results of previous studies.

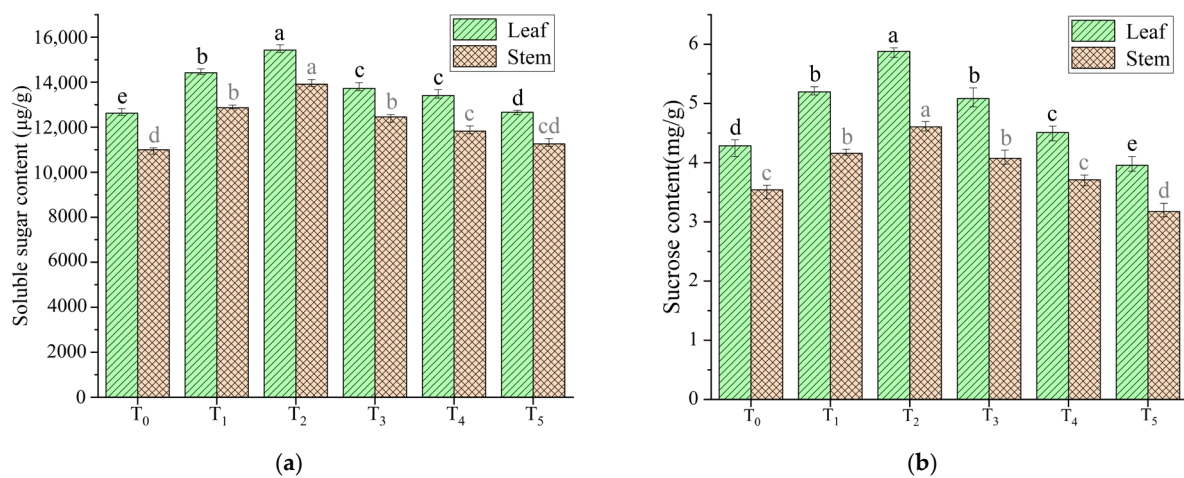


Figure 2. The sugar content of leaf and stem of plug seedlings under different treatments: (a) Soluble sugar content; (b) Sucrose content. The value shown was significant when $p < 0.05$ by the least significant difference (LSD) multiple comparison method as indicated by different letters.

2. N content of leaf

The sugar content of seedlings mainly represented their metabolic capacity and nutrient transport, while the nitrogen content of the leaf was an important reflection of the final nutrient content and growth of seedlings. In order to obtain the accurate nitrogen content of the seedling leaf through spectral technology, it was necessary to establish a relatively optimal and accurate spectral prediction model in advance. Therefore, preprocessed samples were used and divided into two parts: a calibration set and a validation set. Since our research group was familiar with the nitrogen detection method of the seedling leaf and the mature modeling approach, we referred to previous studies and selected a total of 50 samples. The calibration set (30 samples) was used to preliminarily construct the prediction model, while the validation set (20 samples) was used to verify the accuracy and rationality of the model. Finally, the feasibility of the model was evaluated by using the root mean square error $RMSEC$ (including $RMSECV$ calibration of the root mean square error and $RMSEP$ verification of the root mean square error) and correlation coefficient R (Equation (8)): it was better that the $RMSEC$ value was smaller, and it was better that the R value was closer to 1.

$$\begin{cases} RMSEC = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2} \\ R = \sqrt{1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}} \end{cases} \quad (8)$$

where R_C and R_P are the correlation coefficients of $RMSECV$ and $RMSEP$, respectively; y_i and $\hat{y}_i$ are the measured value and predictive value of the i -th sample in the dataset, respectively; n is the total number of samples in the dataset; and $\bar{y}_i$ is the average of the measured values of all samples in the dataset.

Spectral data represented the absorption and reflectance of the seedling leaf to different wavelength spectra, so the measured red edge reflectance of the leaf ($R_{750/700}$) was used to establish an estimation model for the leaf nitrogen content [32]. The preliminary equation for the model was

$$\text{Leaf N}(\%) = k \times R_{750/700} + b \quad (9)$$

where $R_{750/700}$ is the ratio of leaf reflectance at the 750 nm and 700 nm wavelengths.

As shown in Figure 3a, a preliminary prediction model for the nitrogen content of the seedling leaf was established by using 30 calibration set samples and corresponding red edge reflectance parameters: $\text{Leaf N}(\%) = 2.44 \times R_{750/700} + 0.17$, $R^2 = 0.838$, and $RMSECV = 0.48263\%$, $R_C = 0.86639$. The remaining 20 validation set samples were used to validate the established prediction model, and the scatter plot of the measured and predicted nitrogen content values in the validation set is shown in Figure 3b, in which $RMSEP = 0.52104\%$, $R_P = 0.83992$. The results indicated that the previously established prediction model was eligible for test with a good predictive effect on the nitrogen content of the seedling leaf under the same cultivation method.

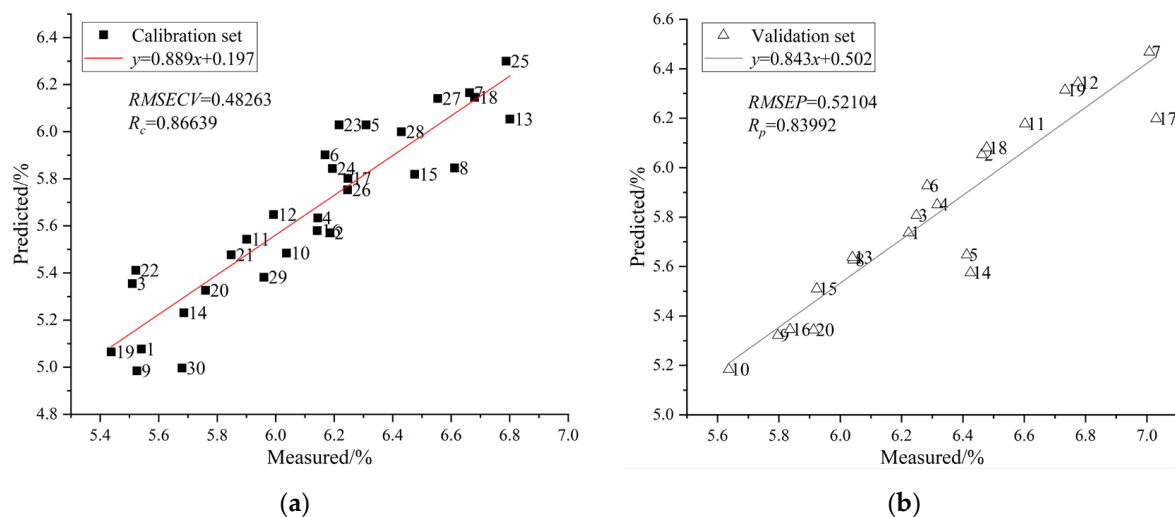


Figure 3. The calibration set and validation set scatter plot of leaf nitrogen content: (a) Calibration set; (b) Validation set.

As shown in Figure 4, the spectral values of the seedling leaves cultivated under different treatments were measured, and the nitrogen contents of different leaves were derived based on the model which was shown in Table 2. The results showed that 10% biochar addition into the substrate significantly increased the nitrogen content (nutrient composition) of the seedling leaf, since the sugar content of the seedling leaf was also the highest under this treatment, which resulted in a significant improvement of nutrient transport capacity and storage in seedlings, so the seedling growth was better with a better nutrient value production. In addition, although the leaf nitrogen content of seedlings under the T_1 and T_3 treatments was lower than that of the T_2 treatment, it was much higher than those under the other treatments. However, the leaf nitrogen content of seedlings under the T_4 treatment showed a significant decreasing trend compared with those under the other treatments, and the leaf nitrogen content of seedlings under the T_5 treatment was surprisingly lower than that of the T_0 treatment, and the reason was that the sugar content of seedling under the T_4 and T_5 treatments was low, which led to the insufficient transportation of nutrients.

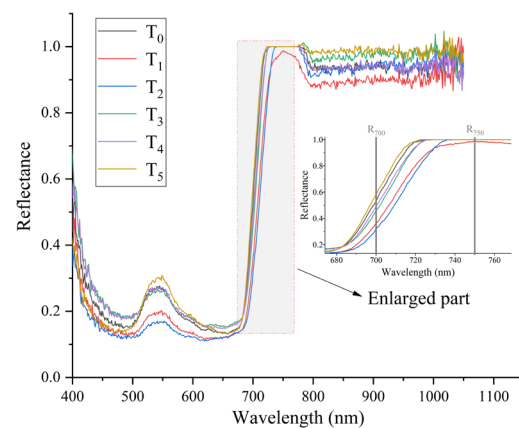


Figure 4. Reflectance of seedling leaves cultivated under different treatments.

Table 2. The leaf nitrogen content of seedlings cultivated under different treatments.

Parameters	Treatments					
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
R ₇₅₀	1	0.9883	1	1	1	1
R ₇₀₀	0.4615	0.3801	0.3518	0.4092	0.4340	0.4723
R _{750/700}	2.1667	2.6002	2.8422	2.4435	2.3043	2.1173
Leaf N (%)	5.46 de ¹	6.51 b	7.10 a	6.13 c	5.79 d	5.34 e

¹ The data in this table were calculated as the average value of different substrate treatments, and the value was significant when $p < 0.05$ by the least significant difference (LSD) multiple comparison method as indicated by different letters in the same column.

To sum up, a specific ratio of biochar in the substrate could significantly improve the seedling growth, with an increase in their sugar content, so the nutrient transport capacity of the seedlings was enhanced, which led to the improvement of the physiological indicators such as the nitrogen content of the seedlings, and the seedlings would achieve better growth and stronger resistance.

3.3. Analysis of Seedling Root Systems Under Different Treatments

3.3.1. Basic Parameters

The total length, average diameter, and total root tips data of seedlings under different treatments are shown in Table 3, and the results indicate that the addition of 10% biochar into the substrate could also achieve the longest total length and total root tips, and these parameters of seedlings cultivated under the T₁ and T₃ treatments were basically the same, but much lower than those of the T₂ treatment. In addition, compared with the other treatments, the root parameters of seedlings cultivated under the T₅ treatment substrate were the worst.

Table 3. The basic parameters of seedling root systems cultivated under different treatments.

Parameters	Treatments					
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Total length/(mm)	117.13 c ¹	131.69 b	143.54 a	130.34 b	119.85 c	102.84 d
Avg diameter/(mm)	0.75 b	0.63 cd	0.59 d	0.66 c	0.73 b	0.8 a
Total root tips	845 d	1034 b	1162 a	1004 b	907 c	785 e

¹ The data in this table were calculated as the average value of different substrate treatments, and the value was significant when $p < 0.05$ by the least significant difference (LSD) multiple comparison method as indicated by different letters in the same column.

A strong root system was a prerequisite for rapid seedling development, since the total length of seedlings under the T₂ treatment was the best, and the root could contact

deeper soil layers; meanwhile, the more root tips there were, the more roots would grow, so the seedling could achieve a greater contact area between the root system and the soil, the seedlings under this treatment and obtain the ability to extract more nutrients from the substrate, so physiological indicators such as the sugar content in seedlings were more abundant, which led to the optimal growth trend of the seedlings. In addition, the root system under the T_2 treatment had the largest number of root tips, and the diameters of these numerous roots (apart from the main root and a small number of lateral roots) which could be described as fibrous roots were usually very small, so the average diameter of this root system was the smallest compared to the root systems under the other treatments.

3.3.2. Lignin, Cellulose, and Hemicellulose Content

As shown in Figure 5, the cellulose and lignin content of the root system first increased and then significantly decreased with the increase in the biochar ratio in the substrates, while the hemicellulose content of the root system first decreased and then increased with the increase in the biochar ratio, and the cellulose and lignin content of the root system was the highest, while the hemicellulose content was the lowest when the biochar ratio in the substrate was 10% (T_2). Furthermore, this trend was consistent with the diameter variation trend of the root system.

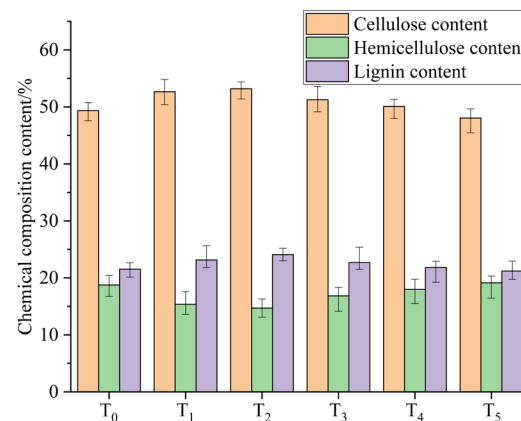


Figure 5. The chemical composition content of root system cultivated under different treatments.

The cellulose and lignin content of the root system cultivated under the T_2 treatment was higher than that of other treatments, and the reason was that the addition of 10% biochar into the substrate could significantly promote the root system growth, and the seedlings tended to increase the size of root epidermal cells when the average root diameter decreased significantly. At the same time, the distribution of photosynthetic products to the root system decreased when the nutrient supply of seedling was sufficient, so the main root in the root system would produce more meristematic tissues, and the number of roots (especially the fibrous roots) would significantly increase, which led to a significantly increase in the cellulose and lignin content of the root system [33].

3.3.3. Tensile and Shear Resistance Properties

The mechanical properties of the root systems under different treatments were measured after excluding some failed and abnormal data, and the variation in the mechanical properties with the root diameter is shown in Figure 6. The ultimate tensile and shear resistance of the root systems increased exponentially with the root diameter, and $T_5 > T_0 > T_4 > T_3 > T_1 > T_2$, while the tensile and shear strength decreased exponentially with diameter, and $T_2 > T_1 > T_3 > T_4 > T_0 > T_5$. Since the root system under the T_2 treatment had a large number of fine roots, and these fine roots had a poor tensile and shear resistance, this led to the poor average tensile and shear resistance of the root systems.

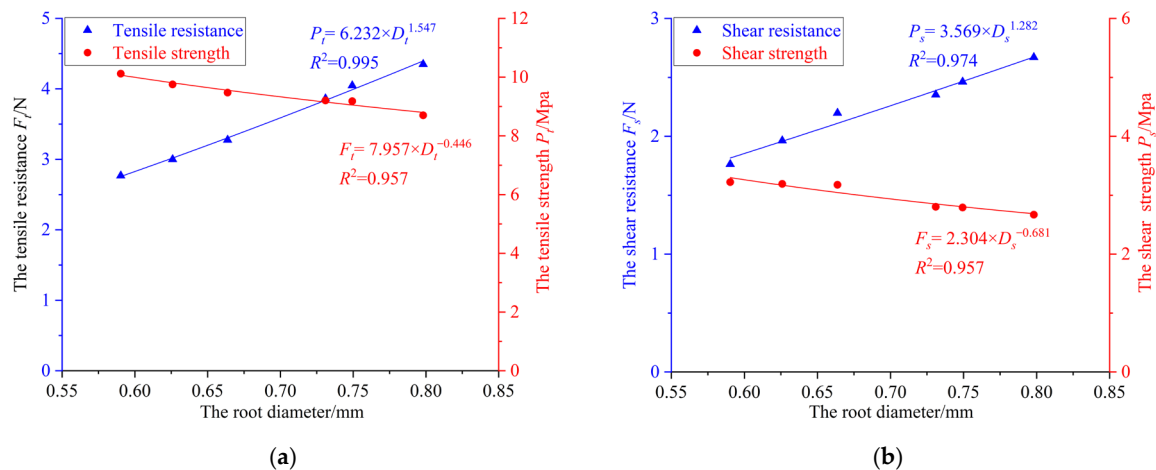


Figure 6. The mechanical properties of root system under different treatments: (a) Tensile properties of root systems; (b) Shear properties of root systems.

Previous research has indicated that cellulose with a strong stability is the most important component of seedling root cell walls, which directly affects the development of the root tissue structure and then the mechanical properties of the root system. As the main filling and bonding component in the cell wall of the xylem, lignin could make the cellulose bond more tightly, thereby enhancing the mechanical properties of the root system. However, the hemicellulose content had little effect on the mechanical properties of the root systems [34]. Since the cellulose and lignin content in the root system decreased with the increasing of the root diameter, the tensile and shear strength significantly decreased similarly. However, the root system under the T_2 treatment had the largest number of roots besides the main root, and the diameter of these fibrous roots was usually very small, resulting in the smallest average diameter with a highest cellulose and lignin content in the root system compared with the other treatments, so the tensile and shear strength of the root systems under this treatment achieved the highest value.

3.4. Analysis of Mechanical Property of the Seedling Pots Under Different Treatments

3.4.1. Compression Resistance Property

As shown in Figure 7a, the addition of biochar into the substrate significantly affected the compression resistance property of seedling pots, which achieved the highest value (49.52 N) under the T_2 treatment and lowest value (33.9 N) under the T_5 treatment (33.9 N).

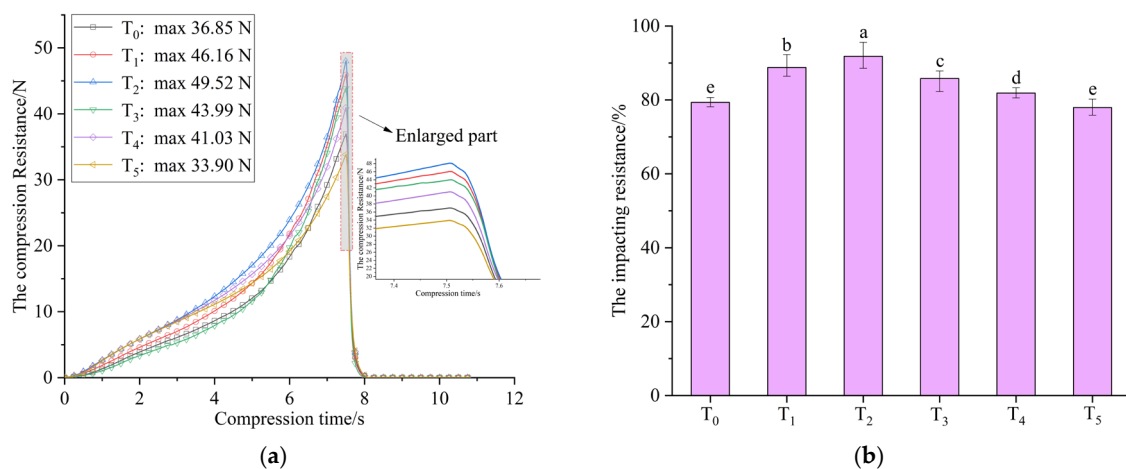


Figure 7. The mechanical property of seedling pots cultivated under different treatments: (a) The compression resistance; (b) The impacting resistance. The value shown was significant when $p < 0.05$ by the least significant difference (LSD) multiple comparison method as indicated by different letters.

As a carbonized product, the biochar had a good mechanical property, so in theory, the higher the proportion of biochar in the substrate, the better the mechanical properties of the seedling pots. However, this theory did not apply to the seedling pot, which was a complex of the root system and the substrate. The higher the proportion of biochar in the substrate, the worse the root systems of the seedling pots, and since the mechanical property of the seedling pot was the result of the interaction between the root system and the substrate, the higher biochar ratio led to a worse mechanical property, and the compression resistance of seedling pots under the T_5 treatment (33.9 N) was even lower than that of the T_0 treatment (36.85 N). The difference in the mechanical properties of seedling pots under the T_1 treatment (46.16 N) was relatively small compared to that of the T_2 treatment (49.52 N), and the reason was that the seedlings cultivated under the T_1 treatment also obtained relatively strong root systems, so the substrate could also be wrapped tightly compared with those under the other treatments, which led to a relatively good mechanical property. The root systems of seedlings were poor when the proportion of biochar in the substrate exceeded 15%, so the root systems could not wrap the substrate tightly, resulting in the poor mechanical properties of these seedlings. Therefore, the proportion of biochar added into the substrate should be appropriate.

3.4.2. Impacting Resistance Property

As shown in Figure 7b, the impacting resistance of the seedling pot was similar to its compression resistance, and the seedling pot cultivated under the T_2 treatment resulted in the lowest degree of damage (91.78%) and the highest degree of damage (77.9%) was seen under the T_5 treatment.

The damage degree of seedling pots after impacting is shown in Figure 8, which indicates that the probability of damage to the areas without root growth was relatively high after impacting, and the damage was more serious. Thus, the degree of root system development in the seedlings was an important factor which determined the mechanical properties of the seedling pot. The seedlings under the T_2 treatment had the strongest root system, with the most roots and the longest total length, so the root system under this treatment could deeply penetrate and tightly wrap the substrate, and a large number of fibrous roots had good tensile and shear properties, which resulted in the best mechanical property [11], and the seedling pots could maintain maximum completeness under this treatment. Although the biochar addition in the T_5 treatment was the greatest and the substrate itself had a good mechanical property, the root systems under this treatment achieved the worst value, with the shortest length and the least root tips, so the substrate of this treatment was loosened and could not be wrapped by the root system, which led to a poor mechanical property. Thus, the strong root systems brought about by the appropriate proportion of biochar into the substrate were a prerequisite for ensuring good mechanical properties.

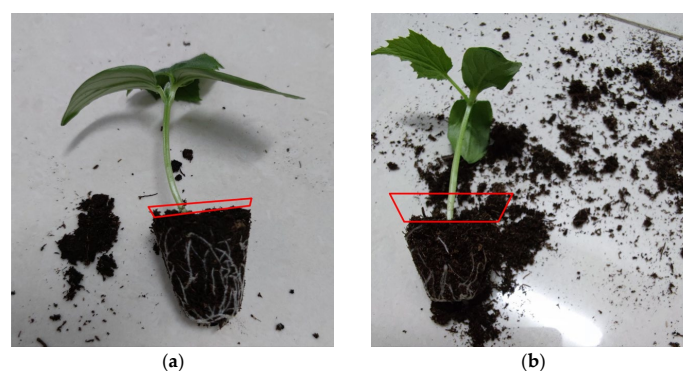


Figure 8. The damage degree of seedling pots after impacting: (a) Small damage degree; (b) Large damage degree. Notes: The red boxed area in the figure was the damaged area where the seedling pots were impacted.

3.5. Analysis of Leaf Gas Exchange Parameters of the Seedlings Cultivated After Impacting

The results of the leaf gas exchange parameters of the seedlings cultivated after impacting are shown in Table 4, and these parameters basically increased first and then decreased with the increase in the biochar ratio in the substrate, and the parameters under the T₁ and T₂ treatments were almost the same during the growth stage, but the vast majority of these parameter values were still optimal under the T₂ treatment.

Table 4. The leaf gas exchange parameters of the seedlings which cultivated after impacting under different treatments.

Parameters	Treatments					
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Net photosynthetic rate/ μmol (CO ₂) m ⁻² s ⁻¹	13.838 d ¹	15.632 a	15.862 a	14.928 b	14.293 c	13.32 e
Stomatal conductance/ mmol (H ₂ O) m ⁻² s ⁻¹	0.859 c	1.073 a	1.109 a	0.95 b	0.914 b	0.812 d
Intercellular CO ₂ concentration/ μmol (CO ₂) mol ⁻¹	184.663 d	205.121 a	207.714 a	197.322 b	190.593 c	180.95 e
Transpiration rate/ mmol (H ₂ O) m ⁻² s ⁻¹	9.533 e	11.294 a	10.915 b	10.424 c	10.13 d	9.324 e

¹ The data in this table were calculated as the average value of different substrate treatments, and the value was significant when $p < 0.05$ by the least significant difference (LSD) multiple comparison method as indicated by different letters in the same column.

The results indicated that the porosity and water-holding capacity of the substrate were both at an optimal value when the addition of biochar ratio into the substrate was 5%~10%, which could cultivate seedlings with strong root systems and large leaf areas. Strong root systems guaranteed the good mechanical propriety of seedling pots, which resulted in a high degree of integrity of the seedling pots and minor damage of the root systems after impacting, so the seedlings could grow very well with the sufficient nutrient supply and initial larger leaf areas, and the leaf gas exchange parameters of the seedlings would achieve the best value compared with the other treatments. As the proportion of biochar ratio in the substrates continued to increase, the seedling pots and root systems were severely damaged after impacting. According to the theory of plant physiology [35], the damage to the root system directly affected the health of seedlings, which would greatly prolong the rejuvenation period of transplanted seedlings; thus, the ability of seedlings to absorb water and nutrients from the soil was poor, and the stomata of the seedling leaves extensively closed due to the poor water and nutrients supply, and then resulted in a decrease trend of seedling photosynthetic rate and transpiration [36], so the seedling growth was inhibited when the biochar ratio in the substrate was excessive. To sum up, when the biochar ratio mixed into the substrate was appropriate, the seedlings would grow rapidly with complete and powerful organ functions, so the seedlings could achieve more effective photosynthesis after transplantation, and thereby the seedling growth after transplanting was significantly promoted.

This study had found a new cultivation method for plug seedlings, which could meet automatic transplanting requirements. However, this study only investigated the cultivation of cucumber plug seedlings due to time and cost constraints, and we should continue to investigate whether this method is applicable to other different vegetables. Moreover, the influence of seedlings under different biochar preparation materials and methods can also be considered as follow-up research.

4. Conclusions

Based on the advantages of biochar for crop cultivation, this study demonstrated the effects of the seedlings on the growth trend, root systems, mechanical properties before transplanting, and the leaf gas exchange parameters after transplanting with biochar added

into the substrates. The mixed substrate could meet the requirements for cultivating plug seedlings. The basic growth parameters and physiological indices of the plug seedlings firstly increased and then significantly decreased with the increasing of biochar ratio in the substrates, which achieved the highest value when the biochar ratio was 10% in the substrate, but the seedlings achieved the lowest value with 25% biochar treatment, even lower than that of 0% biochar treatment. The clear growth trend provided strong root systems of the seedlings, including a longer root length and a better tensile and shear strength, so the substrates under this treatment could be wrapped tightly, which resulted in the optimal compression resistance properties of the seedling pots. However, the compression resistance properties of seedlings under 20%, 25%, and 0% treatments were much lower than the seedlings cultivated under 10% treatment. The seedling pots under 10% biochar treatment maintained maximum integrity compared with the other biochar treatments, so the seedlings could grow well during the flower stage even when the seedlings were impacted after free dropping. However, the seedling pots suffered significant damage after impacting when the biochar ratio in the substrates was beyond 15%, with a certain extent of damage to the root systems at the same time, so the nutrient and water absorption of the seedlings under these treatments was hindered, and these seedlings could not grow well after transplanting. To sum up, a specific suitable ratio of biochar in the substrate could not only promote the growth of plug seedlings but also enhance the mechanical properties of the seedling pots, and these seedlings could also achieve a good growth trend after transplanting, which would have a good application prospect in the automatic transplanting industry.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture14112012/s1>, Figure S1: The measurements of physiological indices of seedlings under different treatments; Figure S2: The basic root system growth parameters of seedlings under different treatments; Figure S3: The mechanical properties of root systems and seedling pots of seedlings under different treatments.

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