Two-stage seedling cultivation method for sweet peppers combining closed plant factory and solar greenhouse

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Abstract: This study introduced a two-stage cultivation method for sweet pepper seedlings, integrating the strengths of a closed plant factory and solar greenhouse, to mitigate the environmental constraints in Northeast China during the early spring season. In the first stage, seedlings were cultivated in a closed plant factory, followed by a second stage in a solar greenhouse. Four treatments- T1 (9 and 36 d), T2 (12 and 33 d), T3 (15 and 30 d), and T4 (18 and 27 d) - were designed for the first and second stages, respectively, with solar greenhouse-only approach serving as the control (CK). The findings reveal that the two-stage methodology significantly outperformed the control across multiple metrics, including seedling health index, chlorophyll content, photosynthetic capacity, yield, etc. Specifically, T3 emerged as optimal, boosting the health index by 38.59%, elevating chlorophyll content by 39.61%, increasing net photosynthesis by 34.61%, and augmenting yield per plant by 40.67%. Additionally, T3 expedited the time to harvest by 25 d compared to the control. Although the seedling cost for T3 was 0.12 RMB yuan higher, the benefits offset the additional investment. In conclusion, the two-stage cultivation method effectively leverages the advantages of both closed-plant factories and solar greenhouses, resulting in superior seedling quality compared to using only solar greenhouses. It offers a practical and economically viable solution for enhancing the quality and yield of sweet pepper seedlings, thus contributing to the progress in the field of facility seedling cultivation research.

Keywords: two-stage, seedling cultivation, sweet peppers, closed plant factory, solar greenhouse
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1 Introduction

Sweet pepper (*Capsicum annuum* L.) is a member of the Solanaceae family and is one of the most fundamental foods in daily diets, making its supply essential for maintaining the livelihood of people[1-3]. The seedling cultivation of sweet pepper represents a critical phase in the production process, with the pursuit of producing high-quality and robust seedlings being the primary objective of the seedling cultivation industry[4]. Seedling quality directly influences sweet pepper production management, yield quality, and market launch timing, which subsequently affects the income of sweet pepper growers and the development of the sweet pepper industry[5].

Temperature and light are the most important environmental factors affecting the growth and development of sweet pepper seedlings. Especially in the early stage of seedling[6,7], low temperature and short daylight hours severely limit the development of the seedlings[8,9]. In Northeast China, the relatively suitable planting season (from April to October each year) has been reserved for sweet pepper production due to the relatively short frost-free period. This leads to poor natural environmental conditions during the spring sweet pepper seedling cultivation period (from February to April each year), and they can usually only be cultivated in solar greenhouse[10]. However, subject to natural conditions, in early spring, low temperature and low light (with temperature below 15°C or light intensity below 200 μmol/(m^2·s)) often occur in solar greenhouses, making it difficult to satisfy the environmental conditions required for cultivating high-quality sweet pepper seedlings[11,12]. Therefore, relying solely on natural temperature and light proves inadequate for cultivating superior seedlings[13-15].

Heating and supplemental lighting offer effective solutions to these issues. However, the inherent structural characteristics of solar greenhouses necessitate considerable resource investment to provide the required heating and supplemental lighting for pepper seedling cultivation[16]. Moreover, given the relatively brief seedling period, the installation of supplementary lighting and heating devices not only substantially elevates production costs but also generates detrimental effects on subsequent solar greenhouse production, such as shading. Although closed plant factories provide a completely controlled environment for plant production, enabling the creation of suitable environments for the growth of pepper seedlings, shortening the seedling growth period and improving seedling quality, their high operating costs, high energy consumption, and prolonged seedling recovery time after transplantation limit their widespread adoption in production[17-20].

Consequently, this study aimed to develop a two-stage seedling

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method for sweet peppers, specifically tailored for cold regions in northeast China, based on the physiological and developmental characteristics of the seedlings. The initial stage (the first stage) was planted at high density in closed plant factory since the aim of this study was forming good photographic establishment at the early stage of seedling raising and laying the foundation for raising robust seedlings at the later stage while saving cost as much as possible. In the later stage (the second stage), the seedlings were transplanted into a solar greenhouse, utilizing the natural improvement in temperature and light to produce high-quality seedlings with minimal input costs. Sweet pepper (*Capsicum annuum* L., cv. Jifeng) was chosen as the plant sample for this study, and the growth, development, photosynthetic characteristics, and yield of the sweet pepper plants were evaluated through the observation of the phenological period. Based on the findings, the optimal two-stage seedling breeding program was identified, which resulted in the production of high-quality sweet pepper seedlings with significantly reduced cost investment.

2 Materials and methods

2.1 Experimental materials and site

With sweet pepper (*Capsicum annuum* L., cv. Jifeng) as the cultivation species, the experiment was performed in closed plant factory and solar greenhouse in the Jilin Academy of Vegetable and Flower Science, China (125°23'37.1"E, 43°49'51.9"N) from February 2022 to August 2022.

2.2 Methods

The sweet pepper seeds were soaked for 4 h and subsequently placed in the germination chamber at a temperature of 28°C and a humidity of more than 90% for 2 d to initiate germination. Post-germination, 384 seeds were sown in 128 cell plug trays containing grass charcoal, vermiculite, and perlite (3:1:1 by volume), while 96 seeds were sown in 32 cell plug trays. These were designated as the treatment group and the control group (CK), respectively, and both were placed in the germination chamber. The environment was set as follows: the temperature was (28±1)°C in the photoperiod and (22±1)°C in the dark period, the relative humidity was set as (80±10)%, and the seedlings were irrigated with water every 2 d until emergence.

After germination, the treatment group was placed in closed plant factory for the first stage of seedling development. The environmental temperature was controlled at (25±1)°C in the photoperiod and (18±1)°C in the dark period, the relative humidity was 55%±5% and the concentration of CO_2 was (800±50) μmol/mol. Liu et al.[15] showed that LED lights with a spectral composition R:B ratio of 1.5 were suitable for pepper seedlings in closed plant factory. Therefore, in this study, a plant growth light with R:B ratio of 1.5 was used for the light treatment (Tianjin Jinya Electronics Co., Ltd., China) (Figure 1), and the photoperiod was set from 6:00 to 20:00 with a light intensity of 150 μmol/(m^2·s)). Meanwhile, CK was moved to solar greenhouse, and the environmental parameter was set as follows: the temperature was (22±7)°C in the photoperiod and (15±3)°C in the dark period, the relative humidity was 30%-60% in the photoperiod and 70%-90% in the dark period, and the insulation quilt was opened from 08:00-16:00 and closed at other times.

When the treatment group of sweet pepper seedlings grew in closed plant factory for 9, 12, 15, and 18 d, 32 seedlings were taken from each group and moved to 32 cell plug trays, recorded as T1, T2, T3, and T4, and moved to the same sola greenhouse as Check group (CK) for the second stage of seedling development (Table 1).

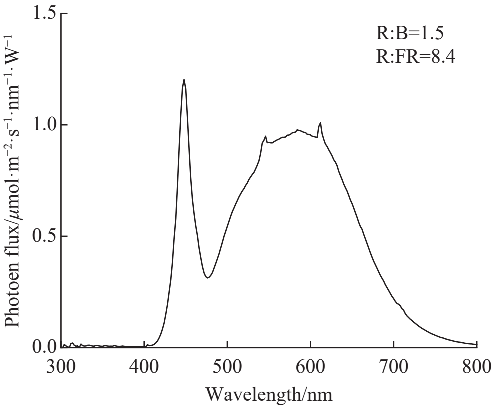


Figure 1 Spectral distribution of plant growth lamps with R:B ratio of 1.5

Table 1 Experimental design

Treatment	Closed plant factory/d	Solar greenhouse/d
CK	0	45
T1	9	36
T2	12	33
T3	15	30
T4	18	27

Note: CK: Check group.

Sweet pepper seedlings were irrigated by bottom irrigation for 10 min each time, which was formulated with Japanese garden-type formula (major element with mol/L in N: P: K: Ca: Mg=16: 4: 8: 8: 4; micro element with mol/L in Fe: B: Mn: Zn: Cu: Mo=3: 0.5: 0.5: 0.05: 0.02: 0.01). During the period from seed emergence to the first true leaf development, the seedlings were irrigated with 1/3 concentration of nutrient solution every 2 d. After the first true leaf development, 2/3 concentration of nutrient solution was used for daily irrigation, and after the second true leaf development, the seedlings were irrigated with a standard concentration of nutrient solution with pH of 6.0-6.5 and EC of 2.0-2.4 mS/cm.

On the 45th day post-emergence, 20 seedlings of uniform growth were selected from each treatment and transplanted into 1.5-gallon round planting pots containing the previously described substrates. The seedlings were cultivated in solar greenhouse and irrigated with 500 mL of standard concentration nutrient solution daily, double-vine grooming, three inflorescences retained per plant, and three fruits retained per inflorescence.

2.3 Measurement indexes and methods for sweet pepper seedlings

Eight seedlings of uniform growth were selected for each treatment for measurement (Table 2).

Table 2 Measurement time of sweet pepper seedlings

Time/d	Treatment				
9	CK	T1			
12	CK	T1	T2		
15	CK	T1	T2	T3	
18	CK	T1	T2	T3	T4
45	CK	T1	T2	T3	T4

Note: The measurement time is calculated from the emergence of the sweet pepper seedlings. The design of CK, T1, T2, T3, and T4 is listed in Table 1.

2.3.1 Plant morphology and growth characteristics

1) Seedling height: the actual height was measured from the media surface to the meristem tip of seedlings with a steel tape ruler in cm;

2) Stem diameter: the middle part of hypocotyl and cotyledon of the seedling were selected to measure the diameter with an electronic vernier caliper in mm;

3) Number of leaves: counting of true leaves longer than 1 cm;

4) Leaf area: each blade was scanned by a scanner (LiDE-300, Canon Ltd., China) for calculating the leaf area through Adobe Photoshop;

5) Above-ground/below-ground fresh weight: the above-ground and below-ground parts of sweet pepper seedlings were separated and the fresh weight of each plant was weighed and recorded with a ten-thousandth electronic balance (ML104/02, Mettler-Toledo Instruments Shanghai Ltd., China) in g;

6) Above-ground/below-ground dry weight: the above-ground and below-ground parts were placed in an oven (BPG-9156A, Shanghai Yiheng Scientific Instruments Co., Ltd., China) at 105°C for 3 h, then the oven temperature was adjusted to 80°C and the plant was dried for 72 h. After cooling to room temperature, the dry weight of each plant was weighed and recorded with electronic balance;

7) Health index: the health index was determined using Equation (1):

Health index=(Stem diameter/Seedling height + Below-ground dry weight/Above-ground dry weight) × Whole plant dry weight (1)

2.3.2 Chlorophyll content

Leaf samples were selected from the corresponding parts of the middle of both sides of the main veins of the 2nd true leaf. Then, 0.1 g of leaf samples were weighed, cut into thin stripes, and transferred into a 10-mL centrifuge tube carefully. After 10 mL of 80% acetone was added, the samples were soaked at 4°C for 48 h. Then, the chlorophyll extracts were determined by UV/VIS spectrophotometer (UV5, Mettler-Toledo Ltd., Switzerland) at 663, 645, and 470 nm wavelength, and chlorophyll content was calculated with Arnon’s equation[21].

2.3.3 Photosynthetic characteristics

The middle of both sides of the main veins of the second true leaf from top to bottom of the plant was selected to determine photosynthetic characteristics and fluorescence parameters with the novel photosynthesis automatic measurement system (Li-6800, Li-Cor Inc., USA) at 9:00-11:00 a.m. The parameters of photosynthesis measurement were as follows: air velocity 500 μmol/s, light intensity 800 μmol/(m^2·s), CO$_2$ concentration 400 μmol/mol, leaf chamber temperature 24°C, and leaf chamber humidity 60%-80%. The measurement indexes included net photosynthetic rate (Pn, μmol/(m^2·s)), stomatal conductance (Gs, mol/(m^2·s)), intercellular CO$_2$ concentration (Ci, μmol/mol), and transpiration rate (Tr, mmol/(m^2·s)).

2.3.4 Measurement of chlorophyll fluorescence parameters

The determination of chlorophyll fluorescence parameters was conducted using a chlorophyll fluorescence imaging system (IMAGIN-PAM, Heinz Waltz, Germany). On the 18th day of pepper seedling growth, after 30 min of dark adaptation at room temperature, the third fully expanded functional leaf was cut with scissors, the protective cover was opened, and it was placed on the measurement platform of the fluorometer and secured with a string to keep the leaf flat. Three regions of interest (AOI) were selected on the leaf using IMAGIN-WIN. Firstly, the measuring light was turned on (with an intensity of 0.1 μmol/(m^2·s)) to obtain the initial fluorescence yield (Fo), then the maximum fluorescence yield (Fm) was measured using saturating pulse light (with an intensity of 2700 μmol/(m^2·s)). Next, the actinic light (with an intensity of 300 μmol/(m^2·s)) was turned on to measure the actual fluorescence yield (F'), the maximum fluorescence yield under light-adapted conditions (Fm'), and the minimum fluorescence yield under light-adapted conditions (Fo'). The maximum photochemical efficiency (Fv/Fm), the actual photosynthetic efficiency of PSII (Y(Ⅱ)), the electron transport rate (ETR), the photochemical quenching coefficient (qP) was calculated and the non-photochemical quenching coefficient (NPQ) was calculated by the above-measured chlorophyll fluorescence parameters.

2.3.5 Leaf histomorphogenesis

On the 18th day post-emergence, leaf samples were selected from the middle of the main veins on both sides of the second true leaf, and the morphological structure of the front surface of the leaves and the characteristics of the stomata on the back were observed by a digital microscope system (VHX-7000, Keyence Ltd., Japan). The stomatal size (length and width) and stomatal density were measured. Three leaf replicates were taken under each treatment, and 10 fields of view were observed randomly for each leaf. Microscopic observation was performed with parameters that included a falling illumination set to a coaxial slice shot, a tilt angle at 0°, and a magnification of 500×.

2.3.6 Power consumption

After each treatment had completed the first stage of seedling, the power consumption was calculated. In this study, the power consumption of the lighting and nutrient circulation systems was measured using a 10A-electricity monitor (DL333501, Deli Group Co., Ltd., China), while the power consumption of the air conditioning was measured using a 16A-electricity monitor (DL333502, Deli Group Co., Ltd., China).

2.4 Measurement indexes and methods for sweet pepper plants

Eight plants of uniform growth were selected from each treatment for measurement.

2.4.1 Phenological period

The phenological stages of the sweet pepper plants were observed for each treatment, including the budding stage, blooming stage, fruiting stage, and first fruit harvesting period.

2.4.2 Yield and quality of sweet pepper fruits

After the fruits were ripe, the single fruit weight and total fruit weight per plant were measured separately, counting 3 ears of fruits (i.e., King pepper, Vice king pepper, and Hitting king pepper).

The fruit quality was determined with the following indicators: soluble sugar content, and VC content. Soluble sugar content was determined with the anthraquinone method[22] and VC content was determined with the molybdenum blue method[23].

2.5 Data processing method

The processing and analysis of experimental data are completed with Microsoft Excel 2021 and SPSS 22.0. Duncan’s method was used to make multiple comparisons at the significant level of 0.05 when using data statistical methods for significance and statistical analysis. All the pictures were obtained by the Origin 2018 software.

3 Results and discussion

3.1 Indexs of growth and development of sweet pepper seedlings

3.1.1 Growth and development

The two-stage seedling method significantly promotes seedling growth, leaf development, and growth rate. On the 45th day of seedling growth, distinct differences in morphological characteristics were observed among the various treatments (Figures 2 and 3). In terms of seedling height, T4 reached the maximum height of 21.3 cm, while T3 followed closely at 19.43 cm, both were significantly higher than CK, by 49.68% and 36.54%, respectively. Regarding stem diameter, all treatments surpassed CK, with T3 exhibiting the thickest stem at 4.90 mm, which was 36.87% greater than CK.

Leaf area, an indicator of leaf quality, plant growth potential, and robustness[24], also varied among treatments. T4 had the largest leaf area of 190.30 cm^2, significantly more than CK. T2 and T3 showed no significant difference, but both were larger than CK by 48.28% and 43.10%, respectively. The two-stage seedling method impacts not only morphology but also the biomass of sweet pepper seedlings. For whole-plant dry weight, every treatment outperformed CK substantially; specifically, T3 and T4 were higher than CK by 58.24% and 63.27%, respectively.

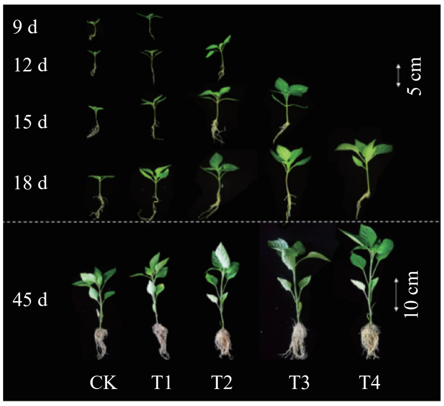
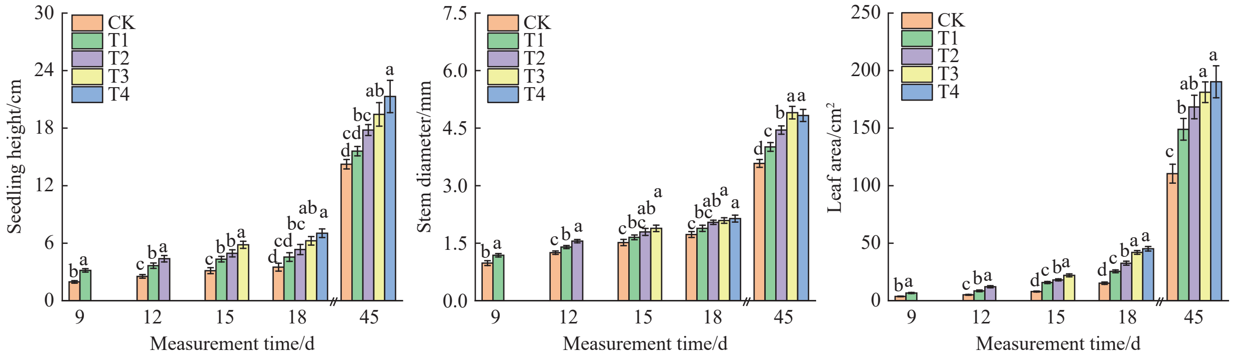
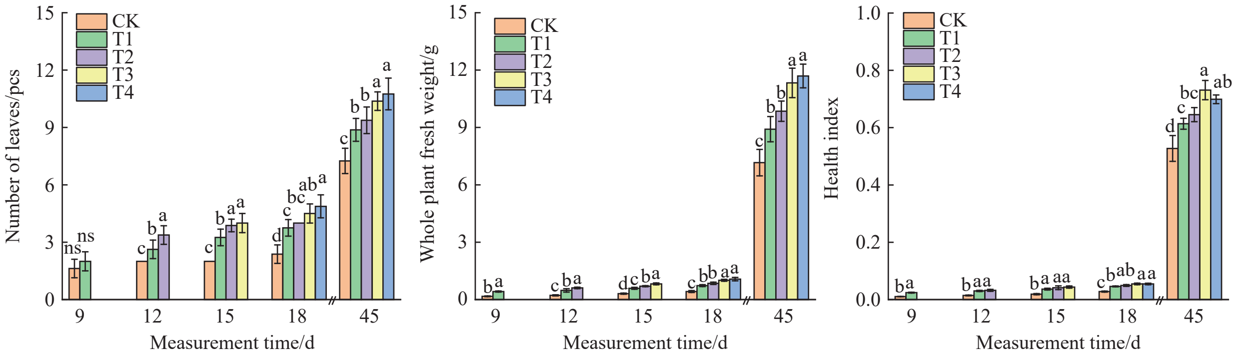


Figure 2 Typical morphology of sweet pepper seedlings



a. Seedling height b. Stem diameter c. Leaf area



d. Number of leaves e. Whole plant fresh weight f. Health index

Note: The measurement time is calculated from the emergence of the pepper seedlings. Different letters in the same column indicate significant differences (p<0.05) based on Duncan's multiple range test. ns represents no significant difference at the 5% level.

Figure 3 Indexs of growth and development of sweet pepper seedlings

The health index serves as a comprehensive measure of the growth quality of sweet pepper seedlings[25]. Short and robust seedlings often have higher health indices, making them more suitable for high-quality vegetable cultivation. In terms of the health index, the ranking was in a descending order of T3, T4, T2, T1, CK (Figure 3f). All treatments significantly outperformed CK, with T3 and T4 being 38.59% and 32.54% higher, respectively.

It is evident that CK, cultivated in solar greenhouse, suffered from severe conditions of low temperature and low light, leading to slower growth and a lower health index. In contrast, the favorable environment of closed plant factory mitigated such stresses, resulting in an improved health index with increased days of growth. However, it is worth noting that a longer cultivation period in closed plant factory does not necessarily yield stronger seedlings. Excessive cultivation time could hinder the ability of seedlings to adapt when transplanted to less favorable conditions in solar greenhouse.

3.1.2 Chlorophyll content

Chlorophyll content serves as an important indicator of the photosynthetic capacity of leaves, with higher content more effectively capturing light energy and increasing the net photosynthetic rate[26,27]. As lited in Table 3, on the 45th day of seedling growth, the chlorophyll a content was highest in T3, followed by T4, T2, T1, and finally CK. T3 had a maximum chlorophyll a content of 2.45 mg/g, which was 36.11% higher than CK. In terms of chlorophyll b, T3 had the highest content at 1.10 mg/g, closely followed by T2 at 1.01 mg/g. Both were significantly higher than CK, showing increases of 46.08% and 33.32%, respectively. No significant difference was found between T1, T4, and CK in terms of chlorophyll b content. As for total chlorophyll content, T3, T2, and T4 showed significant increases compared to CK, with increases of 39.61%, 32.41%, and 25.49%, respectively. Notably, the chlorophyll a/b ratio did not vary significantly across all treatments.

These findings indicate that stress from low temperature and low light reduces overall chlorophyll content without altering its composition, potentially due to the self-regulatory mechanisms within the plant. Furthermore, CK, which was cultivated in solar greenhouse under conditions of low temperature and low light, exhibited a reduction in total chlorophyll content. This decrease was attributed to reductions in both chlorophyll-a and chlorophyll-b levels. This could be due either to inhibited chlorophyll synthesis a loss of chloroplast activity under these stress conditions, or possibly a combination of both.

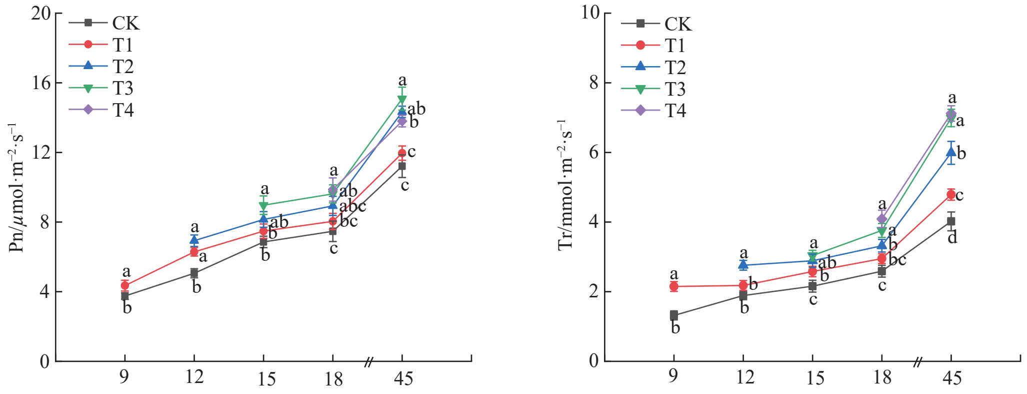
3.1.3 Photosynthetic indicators

Photosynthesis is the sum of a series of complex metabolic reactions and is an important way for plants to accumulate dry matter, and the photosynthetic capacity of leaves determines the rate of photosynthetic product production, which in turn directly affects the yield of fruits[28,29]. Sweet peppers grown for an appropriate duration inside closed plant factory can enhance the photosynthetic capacity of their leaves (Figure 4). On the 45th day of seedling growth, T2, T3, and T4 exhibited significantly higher Pn, Tr, and Gs compared to CK; with the exception of Tr, other photosynthetic parameters of T1 were not significantly different from those of CK, this was possibly due to the fact that they were transplanted to solar greenhouse after a comparatively brief period of cultivation in closed plant factory.

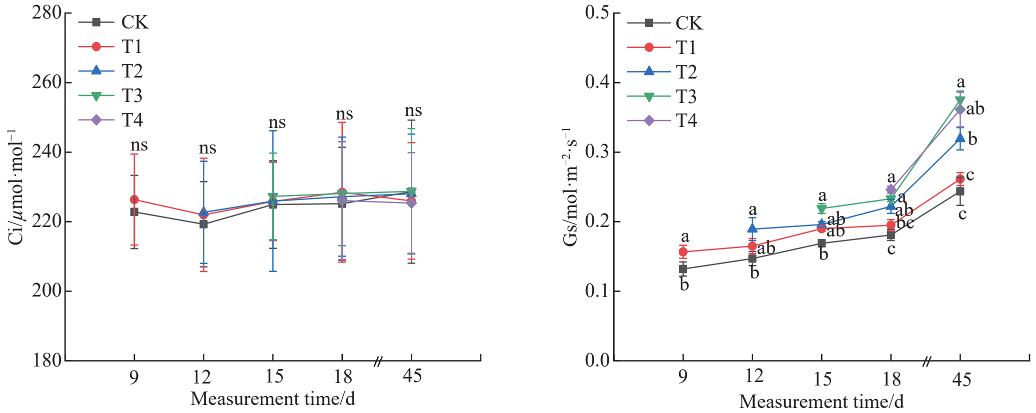
Table 3 Chlorophyll content of sweet pepper seedlings

Measurement time/d	Treatment	Chlorophyll a content /mg·g^{-1}	Chlorophyll b content /mg·g^{-1}	Total chlorophyll content /mg·g^{-1}	Chlorophyll a/b
9	CK	0.85±0.05^{b}	0.39±0.03^{b}	1.24±0.08^{b}	2.20±0.09^{ns}
	T1	1.02±0.05^{a}	0.46±0.03^{a}	1.48±0.08^{a}	2.21±0.07^{ns}
12	CK	0.97±0.05^{b}	0.44±0.04^{b}	1.40±0.09^{b}	2.22±0.10^{ns}
	T1	1.02±0.09^{b}	0.48±0.05^{ab}	1.50±0.14^{b}	2.14±0.08^{ns}
	T2	1.24±0.04^{a}	0.56±0.04^{a}	1.80±0.08^{a}	2.22±0.10^{ns}
15	CK	1.21±0.04^{c}	0.56±0.05^{b}	1.77±0.10^{c}	2.18±0.13^{ns}
	T1	1.41±0.06^{b}	0.63±0.03^{ab}	2.04±0.07^{b}	2.24±0.15^{ns}
	T2	1.39±0.03^{b}	0.63±0.05^{ab}	2.02±0.07^{b}	2.23±0.17^{ns}
	T3	1.60±0.04^{a}	0.73±0.05^{a}	2.32±0.08^{a}	2.21±0.13^{ns}
18	CK	1.37±0.06^{c}	0.61±0.03^{b}	1.98±0.07^{c}	2.23±0.13^{ns}
	T1	1.52±0.07^{bc}	0.70±0.04^{ab}	2.22±0.09^{bc}	2.19±0.17^{ns}
	T2	1.61±0.04^{ab}	0.69±0.03^{ab}	2.30±0.05^{ab}	2.35±0.11^{ns}
	T3	1.75±0.10^{a}	0.78±0.04^{a}	2.53±0.16^{a}	2.23±0.12^{ns}
	T4	1.71±0.08^{a}	0.75±0.07^{ab}	2.46±0.15^{ab}	2.31±0.13^{ns}
45	CK	1.80±0.15^{c}	0.75±0.04^{b}	2.55±0.19^{c}	2.38±0.08^{ns}
	T1	2.05±0.15^{bc}	0.91±0.03^{ab}	2.96±0.18^{bc}	2.26±0.10^{ns}
	T2	2.38±0.17^{ab}	1.01±0.13^{a}	3.39±0.30^{ab}	2.37±0.15^{ns}
	T3	2.45±0.17^{a}	1.10±0.12^{a}	3.56±0.28^{a}	2.24±0.10^{ns}
	T4	2.25±0.11^{ab}	0.95±0.05^{ab}	3.20±0.14^{ab}	2.36±0.12^{ns}

Note: The values listed in the table are mean±standard deviation. Different letters in the same column indicate significant differences (p<0.05) based on Duncan's multiple range test. ns represents no significant difference at the 5% level.



a. Pn b. Tr



c. Ci d. Gs

Note: Different letters in the same column indicate significant differences (p<0.05) based on the Duncan's multiple range test. NS represent no significant difference at the 5% level. Pn is the net photosynthetic rate, μmol/(m^{2}·s); Tr is the transpiration rate, mmol/(m^{2}·s); Ci is the transpiration rate, mmol/(m^{2}·s); Gs is the stomatal conductance, mol/(m^{2}·s).

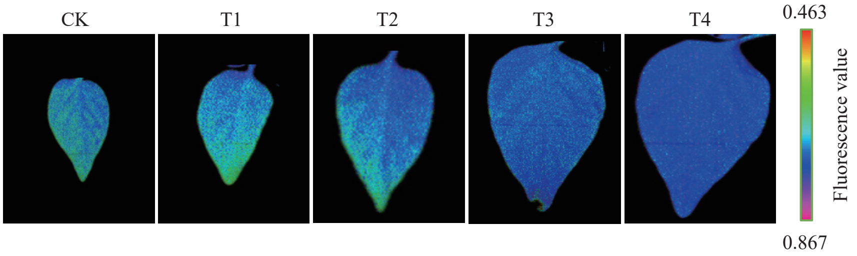
Figure 4 Photosynthetic characteristics of sweet pepper seedlings

In terms of Pn, T4>T3>T2>T1>CK on the 18th day of seedling growth, while T3>T2>T4>T1>CK on the 45th day of seedling growth, Pn of T4 was lower than that of T3 and T2, probably because closed plant factory provided suitable photosynthetic conditions, and transplanting to solar greenhouse might lead to a decrease in photosynthetic capacity by not adapting to the poorer environmental conditions such as lower temperature and lower light of solar greenhouse. On the 45th day, T3 exhibited the highest Pn among all treatments (Figure 4a). This is consistent with the changes in biomass and health index of sweet pepper seedlings. The generation of biomass in sweet pepper seedlings is primarily propelled by the process of photosynthesis, which is reliant on the interception of light. The health index displayed a positive correlation with the dry weight of the plant, thus improving Pn would be advantageous in elevating the quality of sweet pepper seedlings[30].

On the 45th day of seedling growth, T4 and T3 showcased higher Tr, surging by 77.11% and 73.88% compared to CK, which were significantly higher than other treatments (Figure 4b); Gs was higher in T3 and T4, surging by 54.03% and 48.29% higher than CK, without any significant difference between the two (Figure 4d). It is evident that the Tr and Gs were both the lowest in CK. This was attributed to the fact that CK was constantly exposed to an environment of low temperature and low light, resulting in smaller stomatal apertures and lower stomatal densities in the pepper seedlings grown in comparison to those in closed plant factory, thereby directly leading to lower transpiration rates. As for Ci, there was no significant difference among all the treatments, indicating that the differences in photosynthetic characteristics of sweet pepper seedlings were caused by non-stomatal factors such as chloroplasts (Figure 4c).

3.1.4 Chlorophyll fluorescence parameters

Changes in chlorophyll fluorescence serve as indicators of variations in plant photosynthesis[31]. Among these fluorescence parameters, the maximum photochemical efficiency of PSII (Fv/Fm) represents the peak efficiency of light energy conversion at the reaction center[32]. T4 was cultivated in a favorable closed plant factory environment, while T3, T2, and T1 experienced increasing durations in conditions of low temperature and low light. CK was continuously cultivated in solar greenhouse with similar adverse conditions. Fluorescence imaging revealed that as the duration of stress from low temperature and low light increased, Fv/Fm values tended to decline across treatments (Figure 5). This suggests that such stress impedes electron transfer in photosynthesis, obstructing the synthesis of ATP and NADPH and thereby affecting CO_2 assimilation in carbon reactions[33]. The Fv/Fm value in T3 declined less significantly compared to T4, indicating that pepper leaves under T3 conditions maintained higher photosynthetic electron transfer and normal PSII photochemical activity. A significant difference in Fv/Fm between CK and T4 indicates that CK was more adversely affected by the stress conditions of low temperature and low light, causing damage to the PSII reaction center and reducing initial light energy conversion efficiency. Additionally, it was observed that the leaf-tip edges were the initial sites of damage under such stress conditions, and the extent of damage gradually expanded towards the leaf center over time. The leaf veins and leaf bases exhibited comparatively less damage.



Note: Fv/Fm is the maximum photochemical efficiency.

Figure 5 Fv/Fm of sweet pepper seedlings under different treatments on the 18th day of seedling growth

Chlorophyll fluorescence serves as a sensitive and accurate probe for plant photosynthesis, detecting the response of photosynthetic structures to environmental stressors. ETR and Y(Ⅱ) in CK were significantly lower than in T4 and T3, suggesting that stress from low temperature and low light adversely affect normal photosynthesis in sweet pepper leaves (Figures 6a and 6b).

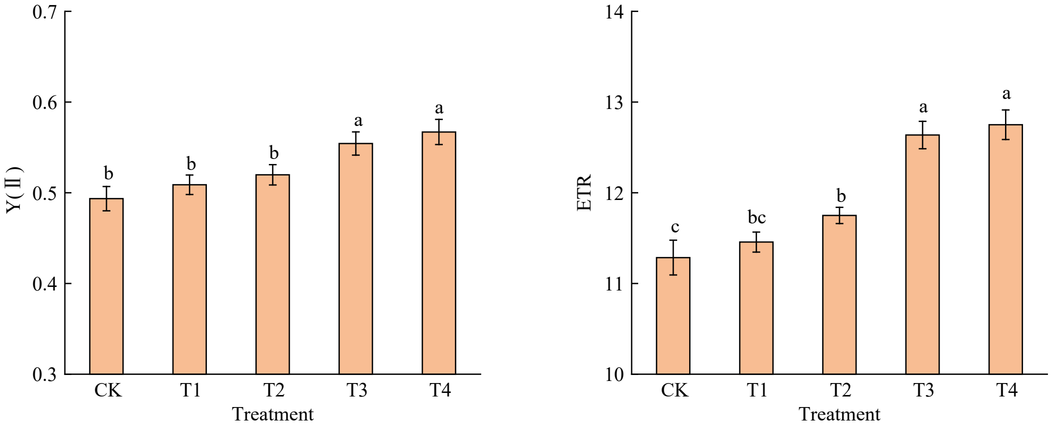
A decrease in ETR implies structural changes in the light-harvesting antenna and PSII reaction center, hindering electron transfer and causing an imbalance between absorbed light energy and metabolic consumption. This results in excessive excitation energy accumulation, leading to PSII photoinhibition. A decrease in Y(Ⅱ) suggests inhibited electron transfer activity, reduced photochemical conversion efficiency, and lowered CO_2 assimilation capacity[33], affecting plant photorespiration and oxygen-dependent electron flow[34]. However, the ETR and Y(Ⅱ) in T3 showed only a slight, non-significant decrease, indicating that its photosynthetic structures were not damaged.

In this experiment, the decrease in qP was not significant in T3 due to the short duration of stress from low temperature and low light (Figure 6c). However, a significant decrease in qP was observed with increased duration of stress, compared to T2, T1, CK, and T4, indicating a disrupted electron transfer chain.

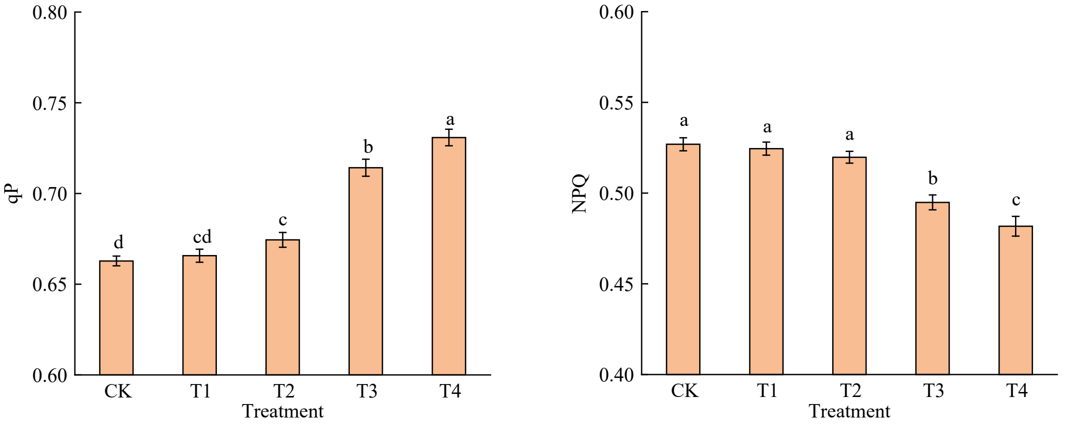
Under conditions of low temperature and low light, sweet pepper seedlings are unable to fully utilize absorbed light energy, causing an imbalance in electron transfer and excess energy. This can stimulate the production of reactive oxygen species, leading to decreased PSII photochemical efficiency and oxidative stress[35]. Various mechanisms are employed within the plant to balance light energy use; non-photochemical quenching (NPQ) is one such mechanism. NPQ dissipates excess light energy, protecting PSII activity[36]. Figure 6 shows that NPQ values increased in the ascending order of T4, T3, T2, T1, CK under low temperature stress, likely due to enhanced violaxanthin de-epoxidase (VDE) activity, facilitating the conversion of violaxanthin to zeaxanthin in the xanthophyll cycle.

3.1.5 Leaf histomorphogenesis

On the 18th day of seedling growth, the microstructure of the leaf surface in sweet pepper seedlings displayed well-defined, irregular, polygonal shapes with raised central tissue and visible stomata, and there were significant differences between treatments (Figure 7). The leaf microstructure in CK appeared more crumpled and desiccated compared to other treatments. As the duration of cultivation in the closed plant factory increased, the folding of surface tissue on sweet pepper leaves gradually diminished. Notably, the unit surface area of tissue in CK was the smallest and most irregularly shaped, while T1 and T2 exhibited relatively small and irregular unit surface tissue areas. In contrast, T3 and T4 had relatively larger and more regularly shaped unit surface tissue areas. One possible explanation for these observations is the superior growth environment provided by closed plant factory compared to solar greenhouse. Additionally, the leaf microstructure in T4 displayed a lighter color, likely due to its cultivation in a closed plant environment with a favorable climate.



a. Y(Ⅱ) b. ETR



c. qP d. NPQ

Note: Different letters in the same column indicate significant differences (p<0.05) based on Duncan's multiple range test. Y(Ⅱ) is the actual photosynthetic efficiency of PSII; ETR is the electron transport rate; qP is the photochemical quenching coefficient; NPQ is the non-photochemical quenching coefficient.

Figure 6 Chlorophyll fluorescence parameters of pepper seedlings under different treatments on the 18th day of seedling growth

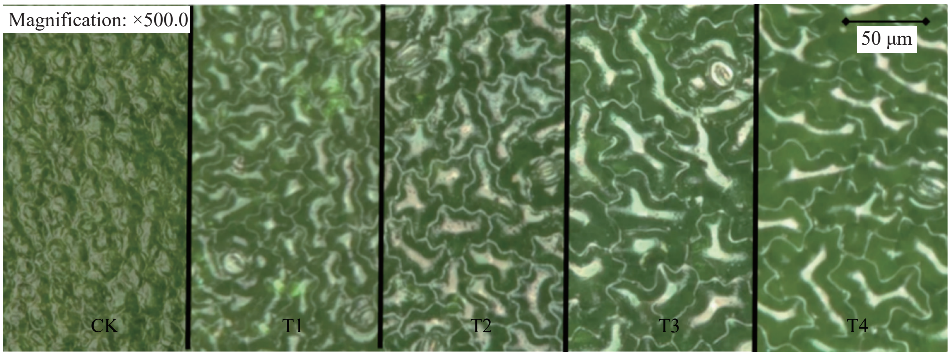


Figure 7 Microscopic tissue structure on the front surface of the sweet pepper seedling leaves on the 18th day of seedling growth

Stomata serve as the primary conduits for the ingress and egress of CO_2 and water in plants[37]. Both stomatal density and size directly influence efficiency in absorbing light quanta and maintaining water metabolic balance in leaves[38,39]. On the 18th day of seedling growth, significant differences in stomatal size were observed among sweet pepper seedlings subjected to various treatments (Table 4). Notably, T4 had the largest stomatal dimensions, exceeding the control by 49.81% in length and 51.52% in width. As the duration of plant growth within the closed plant factory extended, the stomata on the leaves of the pepper seedlings progressively enlarged. In terms of stomatal density, all treatments showed a significant increase compared to the control. There was no significant difference in stomatal density was found between T3 and T4; however, both were higher than the control by 61.27% and 47.64%, respectively. It is evident that stomatal structure is influenced by plant growth environment. Factors such as low temperature and low light in solar greenhouse can affect stomatal development[40]. This could inhibit the carbon assimilation capabilities of sweet pepper seedlings, leading to stomatal unable to self-adjustment for better environmental adaptation. Closed plant factory offer favorable environmental conditions, promoting the healthy development of stomata.

Table 4 Stomatal characteristics on the back of the surface of the sweet pepper seedling leaves on the 18th day of seedling growth

Treatment	Stomatal size/μm		Stomatal density/ number·mm^{-2}
	Length	Width	
CK	20.41±0.17^{e}	13.94± 0.28^{e}	272.08±22.07^{d}
T1	23.29±0.58^{d}	15.61± 0.16^{d}	335.71± 11.69^{c}
T2	26.24±0.37^{c}	17.42± 0.37^{c}	360.11±28.99^{bc}
T3	28.69±0.62^{b}	19.58± 0.22^{b}	438.79±37.78^{a}
T4	30.58±0.42^{a}	21.12± 0.28^{a}	401.71±26.61^{ab}

Note: The values listed in the table are mean±standard deviation. Different letters in the same column indicate significant differences (p<0.05) Based on Duncan's multiple range test.

3.2 Phenological period and yield of sweet pepper plants after transplanting

3.2.1 Phenological period

The growth of seedlings has been demonstrated to influence the timing of flowering and fruiting post-transplantation[41]. The data reveal that the budding stage occurred earliest in T3 (Table 5), ranging from 4.37-17.5 d earlier than other treatments; the latest budding stage was noted in the control group on April 21. Similarly, the blooming stage commenced earliest in T3, with a lead time of 5.87-18.25 d compared to other treatments; T2 and T4 shared the same blooming stage. The fruiting stage also initiated earliest in T3, occurring 5.50-21.63 d before other treatments. Moreover, the first fruit spike was harvested earliest in T3, leading by 9.25-25.25 d compared to other treatments. The shortest duration from planting to the first fruit spike harvest was observed in T3 at 53 d, while the longest was in the control group at 76.75 d. Intriguingly, although T4 exhibited earlier bud emergence than T2, its fruiting period lagged behind that of T2. This discrepancy may be attributed to the extended cultivation period of T4 in closed plant factory, which results in a longer seedling retardation phase upon transfer to solar greenhouse. This observation warrants further investigation to understand the factors slowing down seedling growth when exposed to the challenging conditions of closed plant factory. Overall, our two-stage seedling method significantly impacts the phenological phases of sweet pepper plants.

Table 5 Phenological period of sweet pepper plants

Treatment	Budding stage/d	Blooming stage/d	Fruiting stage/d	First fruit harvesting period/d
CK	25.38±2.12^{a}	33.13±2.71^{a}	41.38±3.24^{a}	78.13±2.71^{a}
T1	22.88±1.76^{b}	29.25±3.73^{b}	36.13±2.85^{b}	76.38±3.49^{a}
T2	15.13±1.69^{c}	20.75±3.34^{c}	25.25±2.17^{d}	62.13±3.06^{c}
T3	7.88±1.36^{e}	14.88±2.89^{d}	19.75±3.10^{e}	52.88±2.32^{d}
T4	12.25±3.07^{d}	21.13±3.92^{c}	30.13±2.89^{c}	66.13±3.14^{b}

Note: The sowing period was on February 5th, and the setting period was on March 28th. The above-mentioned duration is calculated from the setting period. The values listed in the table are mean±standard deviation. Different letters in the same column indicate significant differences (p<0.05) based on Duncan's multiple range test.

3.2.2 Yield and fruit quality of sweet pepper

The yield post-transplantation is heavily influenced by the growth and development of seedlings[42,43]. Since photosynthesis serves as the cornerstone of plant growth and development[44], a higher rate of photosynthesis and CO_2 assimilation implies greater sugar production and dry matter accumulation. This, in turn, provides the material foundation for flower bud differentiation and fruit yield. T3 demonstrated a superior performance in these aspects, showing a larger growth biomass, higher accumulation of dry matter, and an accelerated rate of growth and bud differentiation. Consequently, T3 outperformed the alternative treatments in terms of both single fruit weight and total fruit weight per plant, reaching 245.44 g and 1472.64 g, respectively (Table 6). T1 did not display any significant differences from CK in either single fruit weight or total fruit weight per plant. Although T4 exhibited stronger growth and a higher vigor index during the seedling stage, the total fruit weight for T2 was 4% greater than that for T4 in the later stages. This discrepancy may arise from the extended duration within closed plant factory, which led to excessive vegetative growth at the expense of reproductive growth in sweet peppers, thereby affecting yield.

Table 6 Yield and quality of sweet pepper fruits under different treatments

Treatment	Single fruit weight/g	Total fruit weight per plant/g	Soluble sugar content/%	VC content/ mg·100g^{-1}
CK	174.48±12.27^{c}	1046.88±67.28^{c}	2.87±0.04^{ns}	226.04±3.27^{ns}
T1	190.01±14.70^{bc}	1140.08±72.52^{bc}	2.79±0.07^{ns}	225.94±3.41^{ns}
T2	210.76±13.07^{b}	1304.54±80.68^{b}	2.89±0.04^{ns}	230.26±1.87^{ns}
T3	245.44±21.69^{a}	1472.64±100.14^{a}	2.92±0.05^{ns}	226.39±3.34^{ns}
T4	208.07±11.43^{bc}	1248.42±82.93^{b}	2.85±0.07^{ns}	223.06±2.92^{ns}

Note: The values listed in the table are mean ± standard deviation. Different letters in the same column indicate significant differences (p<0.05) based on Duncan's multiple range test. ns represents no significant difference at the 5% level.

No significant differences were observed in the soluble sugar content or vitamin C content among all the treatments, as shown in Table 6. This suggests that the two-stage seedling method had no adverse impact on fruit quality.

3.3 Costing

The widespread adoption and implementation of closed plant factory are substantially hindered by their high energy consumption and operational costs[45]. These operational costs encompass electricity, depreciation, labor, and miscellaneous expenses such as plug trays, substrates, fertilizers, and seeds[46,47]. Notably, electricity costs constitute the majority of these expenses. In the current study, power consumption was assessed following the completion of the first seedling stage for each treatment. Our findings revealed that costs for artificial lighting accounted for approximately 82% of the total production cost, air conditioning for 15%, and the nutrient solution circulation system for 3%. As the growth period for sweet pepper seedlings in closed plant factory extended, the seedling cultivation cost correspondingly increased (Table 7). According to Kozai[48], enhancing the efficiency of closed plant factory is a viable strategy for reducing production costs. However, existing research suggests that achieving significant performance improvements in closed plant factory in the short term is challenging. To address this, the present study aims to minimize the operational costs in closed plant factory producing sweet pepper seedlings by increasing planting density and reducing cultivation time. Initially, we employed 128-cell plug trays, enhancing space utilization by 300% compared to traditional 32-cell trays. Subsequently, after a 15-day cultivation period in the closed plant factory, seedlings were transferred to solar greenhouse. This considerably lowered the cultivation costs compared to the 36-day period reported by Liu et al.[15]. Concurrently, although the cultivation cost for T3 was 60% higher than that of the control (CK), the fruits reached the market 25 days earlier, yielding profits that far exceeded 0.12 RMB yuan, thus achieving a cost surplus.

Table 7 Cost accounting of different treatments of sweet pepper seedlings

Treatment	Cost in closed plant factory/plant						Cost of solar greenhouse/plant	Total cost/plant
	Electricity consumption		Depreciation	Labor	Others	Total		
	Artificial light source	Environmental control system						
CK	0	0	0	0	0	0	0.2	0.200
T1	0.018	0.004	0.018	0.018	0.015	0.072	0.2	0.272
T2	0.024	0.005	0.024	0.024	0.019	0.096	0.2	0.296
T3	0.030	0.006	0.030	0.030	0.024	0.120	0.2	0.320
T4	0.035	0.008	0.036	0.036	0.029	0.144	0.2	0.345

Note: In this study, the cost of seedlings was calculated according to the method of Katsumi Ohyama[49] (Table 7). Other costs in closed plant factory include expenses for plug trays, substrates, fertilizers, seeds, etc. The cost in solar greenhouse was estimated at 0.2 RMB yuan/plant. The electrical power of the plant growth lights was 18.7 W, the cost of electricity was 0.5 RMB yuan/kWh, and the price of the plug trays was 1 RMB yuan per.

3.4 Correlation analysis and comprehensive evaluation

In order to examine the correlation between the analyzed indices and to conduct a comprehensive evaluation of pepper seedlings grown under different treatments, both Pearson correlation and principal component analysis (PCA) were performed. The Pearson correlation analysis was used to examine the association between each growth and physiological index of pepper seedlings (Figure 8). Positive correlations existed between the health index and seedling height, stem diameter, number of leaves, leaf area, total fresh weight, chlorophyll a content, chlorophyll b content, total chlorophyll content, net photosynthetic rate, stomatal conductance, transpiration rate, Fv/Fm, Y(Ⅱ), ETR, qP, stomatal length, stomatal width, stomatal density, single fruit weight, total fruit weight per plant and total cost. While it was negatively related to NPQ, budding stage, blooming stage, fruiting stage, and first fruit harvesting period. In addition, no correlation was found between the health index and the other examined indicators.

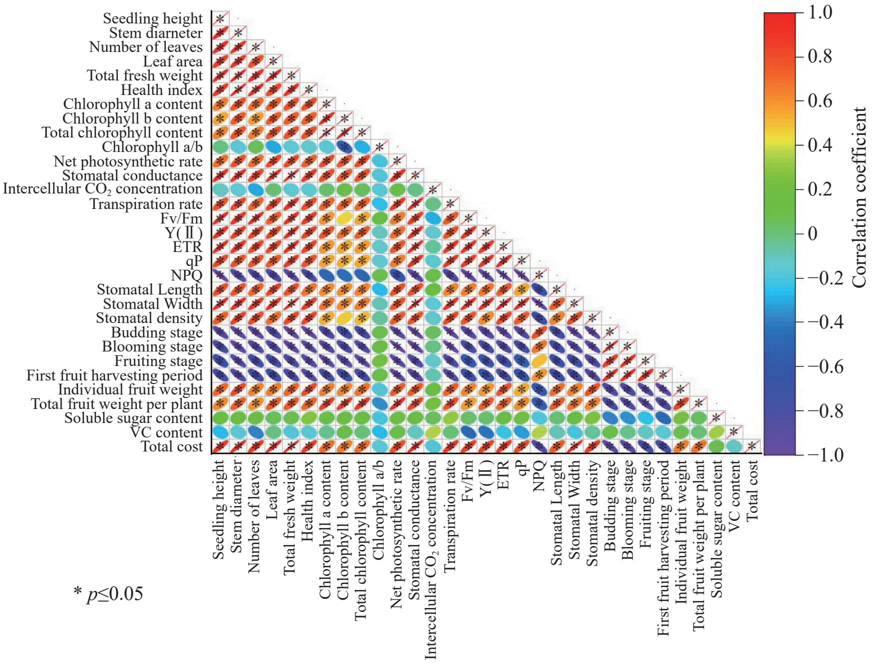


Figure 8 Pearson correlation of each growth and physiological index of sweet pepper seedlings

Principal component analysis (PCA) was used to determine the correlation between each index and treatment, with PC1 explained at 24.20% and PC2 displayed at 18.71% of the total variance (Figure 9). The cumulative contribution rate of 78.9% implies that these two principal components represent a significant proportion of the information in the original data. Consequently, they can effectively achieve the purpose of reducing dimensionality. There was a highly positive correlation between the health index and seedling height, stem diameter, number of leaves, leaf area, total fresh weight, chlorophyll a content, chlorophyll b content, total chlorophyll content, net photosynthetic rate, stomatal conductance, transpiration rate, Fv/Fm, Y(Ⅱ), ETR, qP, stomatal length, stomatal width, stomatal density, single fruit weight, total fruit weight per plant and total cost. Furthermore, the PCA distribution plot indicates clear differentiation among the treatment groups, suggesting significant differences among the various treatments.

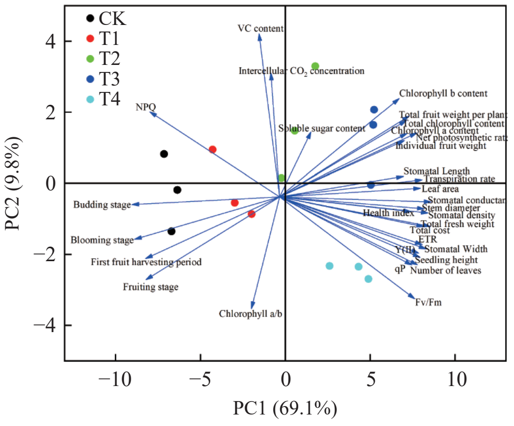


Figure 9 Principal component analysis of each growth index and treatment of seedlings

The SPSS 22.0 software was employed to simplify the evaluated indicators. Subsequently, we formulated a comprehensive scoring model to assess the growth and development of pepper seedlings under different treatments, expressed as $Y=0.691\times Y1+0.098\times Y2$. Utilizing this model, we calculated the comprehensive score value for the growth and quality index of the pepper seedlings, with the following outcomes: CK: −4.673; T1: −2.143; T2: 0.647; T3: 3.683; T4: 2.483. The higher the comprehensive score value, the better the seedling performance under the treatment. Based on the findings, the most effective treatment for the growth and quality of pepper seedlings was observed to be T3>T4>T2>T1>CK.

4 Conclusions

This study investigated the optimal seedling method for sweet pepper plants by utilizing a two-stage cultivation approach involving both closed plant factory and solar greenhouse. Our findings suggest that transferring the seedlings to solar greenhouse following a 15-day cultivation period in closed plant factory is the most effective strategy. This approach significantly minimizes the retardation period during the second stage of seedling development, thus facilitating the cultivation of high-quality, robust seedlings in subsequent stages. Consequently, this leads to an earlier marketing time for the fruit, successfully harmonizing the benefits of closed plant factory with conventional seedling methods. Although this approach incurs a 60% increase in the per-seedling cost compared to conventional methods due to higher initial investments, the resulting seedlings are more robust. This robustness contributes to an earlier fruit marketing time post-transplantation, substantially increasing both yield and production efficiency.

This study focused exclusively on sweet peppers, and in the future, two-stage seedling cultivation research will be conducted on a broader range of vegetable varieties.

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