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Research Article

Influence of cultivation conditions on growth and water use efficiency of zucchini seedlings

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Abstract

With the rapid advancement of facility agriculture, traditional methods of zucchini seedling cultivation face increasing challenges in meeting modern demands for quality and resource efficiency. To address this, a series of experiments were conducted to optimize cultivation conditions aimed at enhancing seedling quality and water use efficiency (WUE). Using the 'Technology No.1' zucchini variety as a model, the study evaluated five substrate formulations, four tray specifications, and five concentrations of an alginate composite agent (ACA) under controlled irrigation based on evapotranspiration data. Key physiological parameters, including plant height, stem diameter, dry matter accumulation, root vitality, and WUE, were measured. The results revealed that a substrate mixture of peat, perlite, and vermiculite (0.7:0.2:0.1) significantly improved plant height (+17.0%), stem diameter (+41.9%), dry matter accumulation (+53.2%), and WUE (+40.6%) compared to the peat-only control. The 50-cell tray configuration provided the most effective balance between root development and spatial efficiency, enhancing root vitality by 8.3%. Among ACA treatments, 150 mg L^{-1} yielded the highest WUE (44.3%) and biomass production, with significantly better performance than both lower and higher concentrations. These findings suggest that the integration of an optimized substrate, tray design, and ACA concentration (peat- perlite - vermiculite substrate + 50-cell tray + 150 mg L^{-1} ACA) can synergistically improve zucchini seedling quality and water efficiency. This study offers a valuable theoretical foundation for the advancement of intensive and efficient seedling cultivation technologies in facility-based zucchini production.

Keywords: alginate composite agent; controlled cultivation conditions; seedling tray design; substrate optimization; water use efficiency; zucchini seedlings

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Introduction

As an important greenhouse vegetable, zucchini occupies a significant position in China's vegetable production in terms of planting area and yield (Yang *et al.*, 2023). With the development of greenhouse agriculture, the seedling cultivation technology of zucchini has gradually shifted from traditional field cultivation to factory and intensive cultivation. However, traditional seedling cultivation methods have many problems, such as uneven growth of seedlings, poor root development, and low water use efficiency (WUE). These problems affect the yield and quality of zucchini and increase production costs. Therefore, exploring better seedling conditions to improve seedling quality and WUE has become an important topic in current agricultural research.

The physical and chemical properties of seedling substrate, as the basic carrier for seedling cultivation, play a key role in the water absorption and growth of plant seedlings. The ideal seedling substrate should have a suitable total porosity (about 70%). The suitable total porosity can ensure that there is enough space in the substrate for air and water to flow freely, providing sufficient oxygen and water for the roots of seedlings, and also promoting the growth and expansion of roots (Ahmed *et al.*, 2024). The pH value should be maintained at around 6.0, which can ensure that the nutrients in the substrate are in the best available state, facilitating the absorption and utilization of seedlings, thereby coordinating water vapor balance and providing stable nutrient supply for seedlings. At present, the "grass charcoal: vermiculite (2:1)" composite matrix has been widely used due to its excellent physicochemical properties. Grass charcoal has good water retention and permeability, while vermiculite can regulate the acidity and alkalinity of the substrate and increase its porosity. The mixture of these two can provide an ideal growth environment for seedlings. However, most existing related studies are limited to observing seedling phenotypes under specific ratios, lacking systematic analysis of substrate physicochemical parameters and plant physiological responses.

The selection of tray specification is also crucial in the process of zucchini seedling cultivation, requiring a comprehensive consideration of economic benefits and biological needs. Although 72 holes tray can improve space utilization, save seedling sites, and reduce seedling costs, it can easily lead to restricted seedling roots and intensified light competition. In a limited space, the root growth of seedlings is restricted and cannot fully extend, thereby affecting their ability to absorb water and nutrients. Meanwhile, too many seedlings crowded together can lead to insufficient light, reduced photosynthetic efficiency, and affect the growth and development of seedlings. Therefore, it is also necessary to further explore the root development characteristics under different tray volumes and their impact on water consumption patterns.

The addition of exogenous substances is an effective way to improve matrix properties. Alginate composite agent (ACA) can improve matrix structure, enhance water and fertilizer retention capacity, promote root development, and improve nutrient utilization efficiency (Sharma *et al.*, 2024). ACA contains abundant components such as alginate, minerals, and microorganisms. These components can improve the physical and chemical properties of the substrate, making it more porous and breathable, while increasing the nutrient content and microbial activity in the substrate. However, most of the current research is focused on field crops, and their application mechanisms in facility seedling cultivation still need to be further explored.

The annual production of zucchini in China accounts for nearly 30% of the world's total production, and its seedling cultivation process is extremely sensitive to water. During the seedling cultivation process, insufficient or excessive water supply can have serious adverse effects on the growth and development of seedlings, such as withering, slow growth, deformities, etc. (Kovačec *et al.*, 2024; Wahyudiningsih *et al.*, 2024). However, there is currently a lack of systematic research on the growth and water use of zucchini seedlings under different seedling conditions. Therefore, based on previous research, this study focuses on key factors such as substrate ratio, tray specification, and ACA concentration, aiming to comprehensively explore the effects of different seedling conditions on the growth and WUE of zucchini seedlings. It is expected to provide theoretical basis and practical guidance for optimizing zucchini seedling cultivation technology and improving WUE.

Materials and Methods

The experiment was conducted at a modern vegetable seedling base of Ningbo Dalong Agricultural Science and Technology Co., Ltd., Ningbo City, Zhejiang Province, China. The planting variety used was zucchini 'Technology No.1'. The experiment started sowing in mid-April 2022, and the experiment continued until mid-July 2022. Growth indicators such as plant height, stem thickness, and leaf number were investigated and statistically analyzed for the zucchini seedlings in each treatment group. Ten seedlings were randomly selected from each treatment group, and their root activity, relative leaf moisture content, and the water use efficiency (WUE) were measured. The average values were calculated to evaluate the effects of different seedling conditions on the growth and WUE of zucchini seedlings. The names, models, and manufacturers of the materials, tools, and drugs required for the entire experiment are shown in Table 1.

Table 1. Statistical table of experimental supplies required

Category	Name	Model/Specification	Manufacturer
Material	Peat	Horticultural grade	China Nonferrous Metal Mining Group
	Perlite	2-4×10^{-3} m particle size	
	Vermiculite	Expanded	
	36 hole disc	Depth 4.144×10^{-2} m, upper diameter 4.933×10^{-2} m, bottom diameter 2.356×10^{-2} m, single hole volume 9.778 × 10^{-5} m^3	Lanling County Xiaoxiao Seedling Equipment Factory
	48 hole disc	Depth 4.144×10^{-2} m, upper diameter 4.933×10^{-2} m, bottom diameter 2.356×10^{-2} m, single hole volume 6.111×10^{-5} m^3	
	50 hole disc	Depth 4.000×10^{-2} m, upper diameter 4.800×10^{-2} m, bottom diameter 2.300×10^{-2} m, single hole volume 5.500×10^{-5} m^3	
	72 hole disc	Depth 4.000×10^{-2} m, upper diameter 4.364 × 10^{-2} m, bottom diameter 1.973 × 10^{-2} m, single hole volume 4.682 × 10^{-5} m^3	
Tool	Electronic scale	Accuracy 1×10^{-4} kg, range 5 kg	Shanghai Precision Instrument Co., Ltd
	Steel ruler	Accuracy 1×10^{-4} m, length 5×10^{-1} m	
	Vernier caliper	Accuracy 1×10^{-5}m, range 0-1.50×10^{-1} m	
	Dry box	Temperature range 50-300 ℃, accuracy ± 1C	
	Electronic balance	Accuracy 1×10^{-5} kg, range 2×10^{-1} kg	
	Measuring cylinder	1×10^{-3} m^3, Division value 1×10^{-5} m^3	
	Spectrophotometer	UV-1800 type, Wavelength range 190-1100 nm	SHIMADZU Corporation
Medicine	Potassium permanganate	Pure analysis, KMnO	China National Pharmaceutical Group Chemical Reagent Co., Ltd
	Avithion	Emulsion concentrates, active ingredient 1.8%	
	Chlorothalonil	Wettable powder, 75%	
	Hogland Nutrient Solution	Standard formula (containing elements such as N, P, K, etc.)	
	ACA	/	
	Phosphate buffer solution	pH7.4	
	Sulfuric acid	Analytical pure	
	Acetic acid mash	Analytical pure	

Experimental design

To investigate the effects of different seedling conditions on the growth and WUE of zucchini seedlings, and to discover the optimal conditions for zucchini seedling growth, a systematic experiment was designed based on the types of seedling substrates, tray specifications, and Alginate composite agent (ACA) concentrations. The specific process of exploring the effects of different substrate ratios in the experiment is shown in Figure 1.

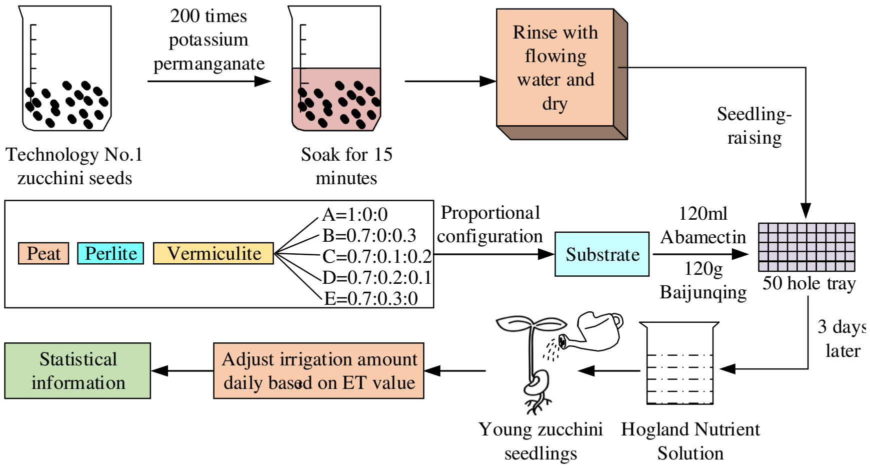


Figure 1. Process of investigating experiments with different substrate ratios

As illustrated in Figure 1, the experimental procedure was systematically divided into five key stages to investigate the influence of substrate formulation, seedling tray specification, and alginate composite agent (ACA) concentration on zucchini seedling development and WUE:

Seed treatment

Zucchini seeds (variety: 'Technology No.1') underwent surface sterilization by soaking in a 200-fold diluted potassium permanganate ($KMnO_4$) solution for 20 minutes. Subsequently, seeds were thoroughly rinsed with distilled water and air-dried in a well-ventilated environment. This disinfection step aimed to reduce microbial interference in the early stages of germination and seedling growth, thereby enhancing the experimental reliability.

Substrate (matrix) preparation

Three standard substrate components-peat, perlite, and vermiculite-were combined in five different volumetric ratios to form experimental treatments:

A = 1:0:0 (peat only); B = 0.7:0:0.3; C = 0.7:0.1:0.2; D = 0.7:0.2:0.1; E = 0.7:0.3:0.

Each cubic meter of prepared substrate was mixed with 120 mL of avermectin and 120 g of chlorothalonil for pre-sowing disinfection, following standard practices for substrate sanitation (Mahmoud *et al.*, 2025).

Sowing and seedling cultivation

Sterilized substrates were filled into the seedling trays, after which one treated and air-dried seed was sown into each cell. Each treatment was replicated five times to ensure statistical validity. Seedling trays were maintained under controlled environmental conditions appropriate for zucchini seedling development.

Nutrient supply

To support uniform seedling development, Hoagland nutrient solution was applied three days post-sowing. This ensured the consistent availability of essential macro- and micronutrients required during the seedling stage (Pandawani *et al.*, 2022).

Water management and irrigation adjustment

Daily water consumption due to transpiration and evaporation was calculated using the weight difference of the seedling trays, measured at 6:00 AM each day using an electronic balance. The daily evapotranspiration (ET) was calculated as:

$ET=W_t-W_{t+1}$= W_t - W_{t+1} (1)

Electronic scales were used to weigh the quality of seedling holes at 6:00 am every day. The calculation of daily transpiration and evaporation of zucchini 'Technology No.1' seedlings is shown in equation 2 (Zou *et al.*, 2024).

$$\gamma(A)=M_1-M_2 \qquad (2)$$

In equation 2, $\gamma(A)$ represents the daily transpiration and evaporation of zucchini 'Technology No.1' seedlings. M_1 is the quality of the seedling hole on that day. M_2 is the quality of the seedling hole the next day. According to the calculation results of equation 2, the irrigation amount of zucchini seedlings under different substrate ratios was adjusted to ensure that the water supply was suitable for the growth of seedlings, and the effect of different substrate ratios on water use was explored. The process of exploring the growth and WUE of zucchini seedlings with different seedling tray specifications is shown in Figure 2.

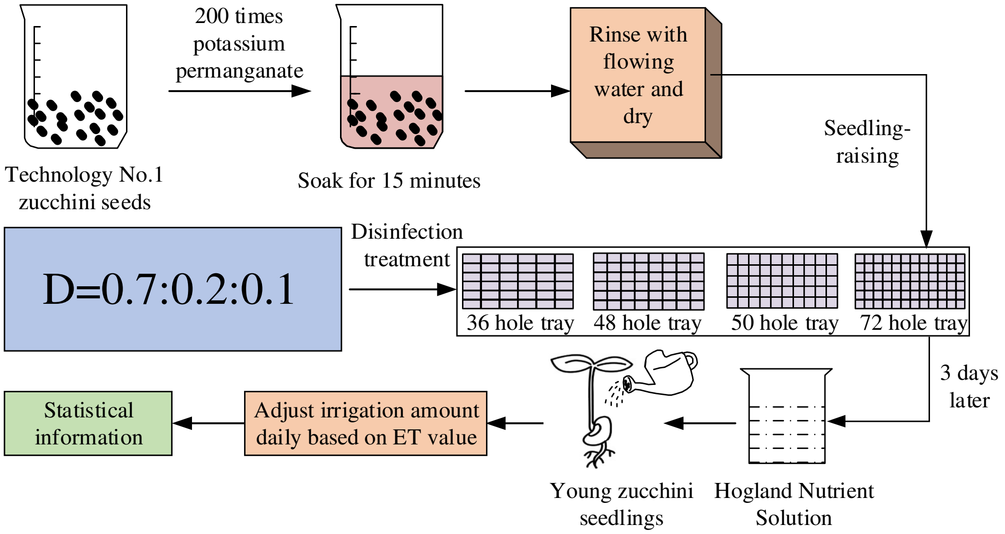


Figure 2. Process of exploring experiments on different seedling tray specifications

In Figure 2, the treatment method and culture substrate disinfection method of zucchini 'Technology No.1' seeds remained unchanged. The substrate with a ratio of D=0.7:0.2:0.1 was placed into trays with 36, 48, 50 and 72 holes. Zucchini 'Technology No.1' seeds were planted into seedling trays with the different specifications, with each substrate treatment set to be repeated 5 times, while other operations remained unchanged. The steps of ACA on the growth and WUE experiment of zucchini seedlings are shown in Figure 3.

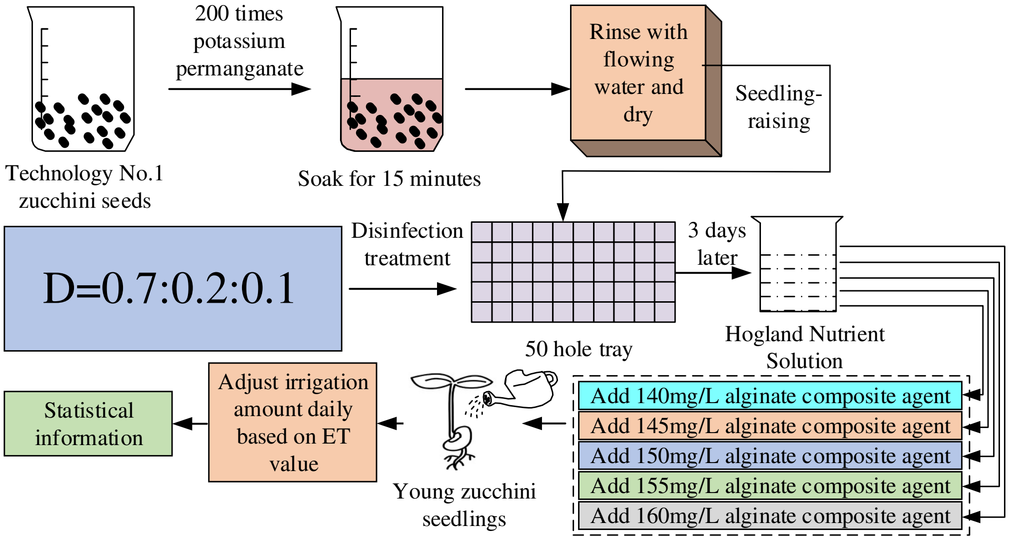


Figure 3. Exploration experiment of ACA at different concentrations

In Figure 3, the treatment method of zucchini 'Technology No.1' seeds and the disinfection method of the culture medium remained unchanged. The substrate with a ratio of D=0.7:0.2:0.1 was filled into a 50 hole tray. Each hole was sown with a single seed and watered with Hogland nutrient solution and different concentrations after 3 days ACA (140 mg L^{-1}, 145 mg L^{-1}, 150 mg L^{-1}, 155 mg L^{-1}, and 160 mg L^{-1}). Similarly, each substrate treatment was set for 5 repetitions, while other operations remained unchanged.

Measurement indicators

Plant height measurement

A steel ruler was used to measure vertically from the cotyledon node to the top of the plant (accurate to 0.01 cm).

Stem thickness measurement

A vernier calliper was used to measure the diameter of the stem at a distance of 1 cm from the ground at the base of the plant (accurate to 0.01 mm).

Number of leaves

The number of fully unfolded leaves on the seedling was directly counted. Leaves were the main organs for photosynthesis, and the number of leaves was related to the photosynthetic capacity of seedlings (Ashton *et al.*, 2024).

Determination of dry matter accumulation

The zucchini seedlings were dug out and washed with distilled water. They were then killed at 105 °C for 30 min and dried in a drying oven at 80 °C to a constant weight. The dry weight was measured using an electronic balance (accurate to 0.01g) (Shi *et al.*, 2024).

Root volume measurement

A measuring cylinder needed to be prepared, an appropriate amount of water should be added, and the initial water level needed to be recorded. The roots of washed zucchini seedlings were completely immersed in water, and the reading was recorded after the water level risen. The difference between two water level readings was the volume of the root system (Wang *et al.*, 2024 a).

Root vitality determination

It was measured using the Triphenyltetrazolium Chloride (TTC) method. A 0.5 g root tip sample of zucchini was weighed and placed in a test tube containing 0.4% TTC solution and phosphate buffer solution.

After a 2-hour dark reaction at 37 °C, 2 mL of sulfuric acid with a concentration of 1 mol L^{-1} was added to terminate the reaction. Then, ethyl acetate extraction was used to extract the red formazan, and the absorbance was measured using a spectrophotometer at a wavelength of 485 nm. The calculation method for root vitality α is shown in equation (3) (Xie *et al.*, 2023).

$$\alpha = \frac{m_{\text{(Onychomyco sis)}}}{m_{\text{(Root fresh weig ht)}} \times T} \tag{3}$$

In equation (3), α represents root vitality, μg TTC/(g·h). $m_{\text{(Onychomycosis)}}$ is the mass of formazan, μg. T is the reaction time. Root vitality reflects the physiological activity of the root system and is closely related to its ability to absorb water and nutrients.

WUE measurement: WUE was calculated based on the daily transpiration and evaporation of zucchini seedlings measured by equation (2), as shown in equation (4) (Ghouil *et al.*, 2024; Kong *et al.*, 2024).

$$\alpha = \frac{m_{\text{(Dry matter accumulation)}}}{\gamma(A)} \tag{4}$$

In equation (4), α is the WUE of zucchini seedlings, g/kg. $m_{\text{(Dry matter accumulation)}}$ is the accumulated amount of dry matter, g.

Data analysis

The data collected during the experiment, including plant height, stem thickness, dry matter accumulation, root activity, relative water content of leaves, and WUE, were organized and statistically analyzed using Excel to calculate the mean ± standard deviation of each treatment group indicator (Sanchez-Lucas *et al.*, 2023). Afterwards, SPSS 26.0 was used for one-way analysis of variance. Taking seedling substrate as an example, the data of plant height, stem diameter, dry matter accumulation, and other indicators of different substrate treatment groups (A, B, C, D, E) were imported into SPSS 26.0 as independent sample sets.

In the "Analysis-Comparison Mean-Single Factor ANOVA" module, the seedling substrate was set as the classification factor, the target indicator was the dependent variable, and the significance level was p=0.05. If the analysis of variance showed significant differences between groups ($p < 0.05$), further Duncan's multiple comparison test would be used to clarify the specific levels of differences between each treatment group (A to E), and the results would be presented in the form of "mean ± standard deviation" (Yang *et al.*, 2025).

Similarly, the analysis of the impact of tray specifications (36 hole, 48 hole, 50 hole, 72 hole) and ACA (140-160 mg L^{-1}) on various indicators followed the same process. The optimization potential of seedling conditions was ultimately evaluated by statistical significance (p-value).

Results

Effect of seedling substrate on the growth and water use efficiency (WUE) of zucchini seedlings

The effects of substrate type on the above-ground morphological traits of zucchini seedlings are shown in Figure 4. ANOVA results indicated that substrate type significantly influenced plant height, stem thickness, and number of blades ($p < 0.05$). Duncan's multiple comparison test further revealed that treatments B and C produced significantly taller plants than treatment A, while treatments D and E showed intermediate values. Similar patterns were observed for stem thickness and leaf number, with significant group differences identified (Figure 4).

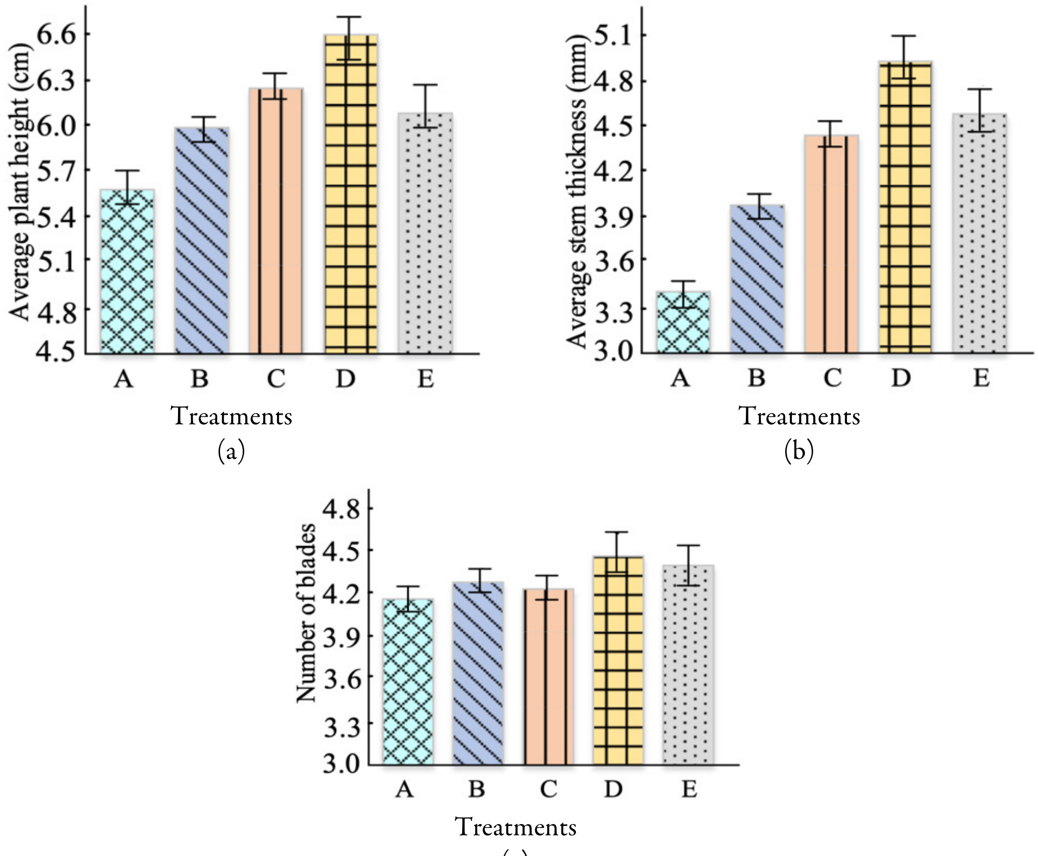
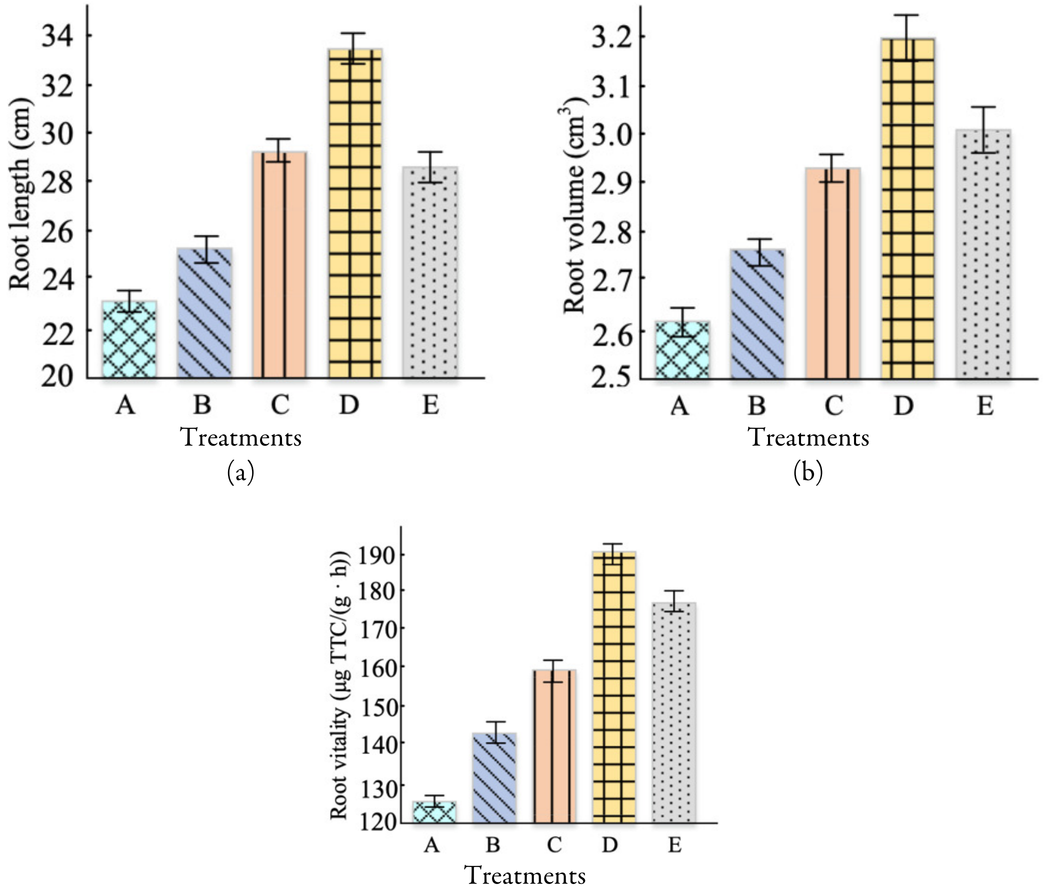


Figure 4. Effects of different substrate treatments (A-E) on the morphological indicators of zucchini seedlings: (a) plant height, (b) stem thickness, and (c) number of blades
Data are presented as mean values with standard deviation bars

In Figure 4 (a), the optimal plant height of D-substrate (0.7:0.2:0.1) was 6.47 cm, which was 17.0% higher than that of pure peat substrate (A-substrate) ($p < 0.05$). This indicated that the addition of perlite and vermiculite in a 2:1 ratio could synergistically improve the substrate structure. Perlite (20%) increased aeration pores, while vermiculite (10%) maintained nutrient buffering through high cation exchange capacity. When the proportion of vermiculite increased to 20%, the plant height of C-substrate (0.7:0.1:0.2) decreased by 3.3%. This was because vermiculite absorbed water and expanded, leading to an increase in bulk density. The plant height of pure peat substrate (A-substrate) was the lowest (5.53 cm), and a single component was prone to compaction, which hinders root extension. In Figure 4 (b), the stem thickness of D-substrate reached 4.84 mm, an increase of 41.9% compared to A-substrate (3.41 mm), indicating the synergistic effect of perlite (20%) and vermiculite (10%): perlite enhanced rhizosphere oxygen diffusion (DO > 6 mg L^{-1}), while vermiculite provided potassium and magnesium ions, jointly promoting vascular bundle development. The stem diameter of B-substrate increased to 4.04 mm, but the plant height was only 5.96 cm, indicating that although 30% vermiculite can improve mineral supply, excessive water retention can inhibit root respiration. In Figure 4 (c), different substrate treatments had little effect on the number of leaves. D-substrate had the highest number of leaves (4.46), only an increase of 3.2% compared to E-substrate with 4.32 leaves. Figure 5 compares the effects of different substrate ratios on the morphological indicators of the underground growth of zucchini seedlings, including root length, root volume, and root vitality.



(a)

(b)

(c)

Figure 5. Effects of different substrate treatments (A-E) on the underground growth indicators of zucchini seedlings: (a) root length, (b) root volume, and (c) root vitality
Data are presented as mean values with standard deviation bars

In Figure 5 (a), the substrate zucchini seedlings in Group D showed the best performance, with a root length of 33.11 ± 0.10 cm, significantly higher than other groups and a 41% increase compared to pure peat (Group A). This indicated that the addition of perlite and vermiculite significantly improved root extension, but excessive perlite (Group E) led to a decrease in length. In Figure 5 (b), the root volume of the substrate zucchini seedlings in Group D ranked first at 3.19 cm^3 ± 0.06, followed by the substrate in Group E. This was because the coarse particle characteristics of perlite could enhance the substrate porosity and promote root extension, but it was also affected by the water retention capacity of vermiculite. In Figure 5 (c), the root vitality of zucchini seedlings in Group D substrate was still the highest. This proved that the D group substrate was the optimal ratio, and the ratio of perlite to vermiculite (2:1) achieved the best balance between improving breathability and water and fertilizer retention, while pure peat or single addition of perlite had obvious shortcomings. Finally, the effects of different substrate ratios on WUE, water consumption, and dry matter accumulation of zucchini seedlings were compared, as shown in Table 2.

Table 2. Dry matter accumulation, water use efficiency (WUE), and water consumption of zucchini seedlings under different substrate compositions

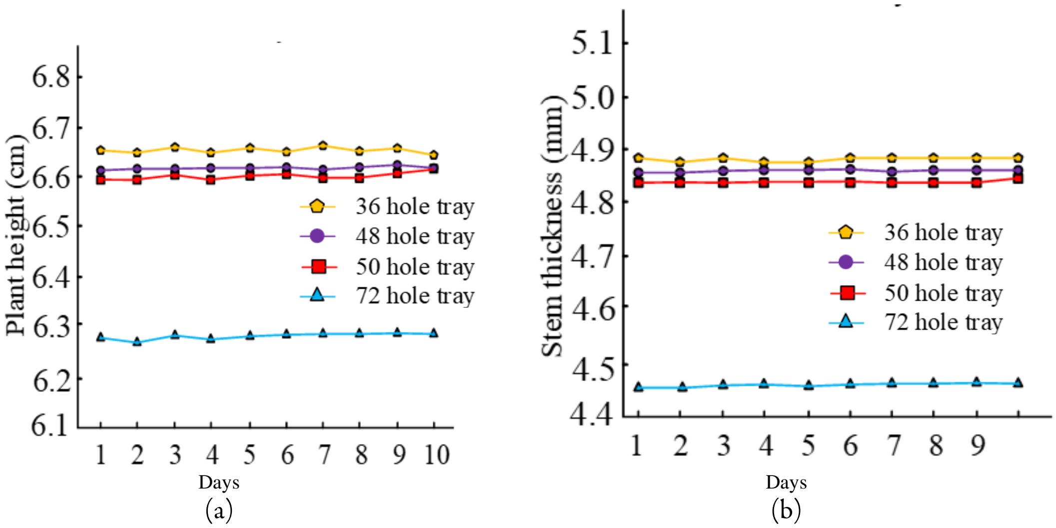
Substrate composition	WUE (%)	Water consumption (mL)	Dry matter accumulation (g)
A	36.41 ± 1.32	335.25 ±5 .63	2.46 ± 0.05
B	41.22 ± 1.29	361.25 ± 6.32	2.81 ± 0.06
C	44.33 ± 1.30	382.36 ± 5.96	3.32 ± 0.05
D	51.22 ± 1.29	410.25 ± 6.24	3.77 ± 0.05
E	46.25 ± 1.32	386.92 ± 6.38	3.54 ± 0.06

Data are presented as mean ± standard deviation

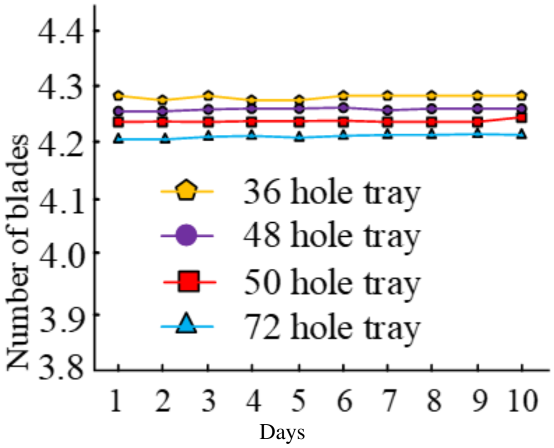
In Table 2, the WUE of zucchini seedlings was highest in D-substrate, at 51.22% ± 1.29%, and significantly higher than other treatments ($p < 0.05$). A-substrate has the lowest value, at 36.41% ± 1.32%. The WUE of zucchini seedlings cultured on D-substrate increased by about 40.6% compared to A-substrate. The water consumption was highest on D-substrate at 410.25 mL and lowest on A-substrate at 335.25 mL. The dry matter accumulation was highest on D-substrate at 3.77 ± 0.05 g and lowest on A-substrate at 2.46 ± 0.05 g. The dry matter accumulation of zucchini seedlings cultured on D-substrate was about 53.2% higher than that on A-substrate. Overall, the substrate ratio showed the best performance in terms of WUE and dry matter accumulation in Group D, while all indicators of pure peat substrate (A-substrate) were significantly lower. This was because adjusting the substrate ratio could maximize the advantages of peat water retention, perlite aeration, and vermiculite-assisted structural stability, achieving a balance of water, air, and fertilizer, thereby significantly improving the WUE and dry matter accumulation of zucchini seedlings.

Effect of seedling tray specification on the growth and WUE of zucchini seedlings

Figure 6 compares the effects of different seedling tray specifications on the morphological indicators of ground growth of zucchini seedlings. In Figure 6 (a), the plant height of zucchini seedlings cultivated using 36 hole, 48 hole, and 50 hole trays remained stable within the ideal range of 6.6 cm-6.7 cm. When using a 72 hole tray, the plant height significantly decreased to 6.3 cm (a decrease of 4.5%). The stem thickness index showed a similar trend, as shown in Figure 6 (b). The stem thickness of the first three types of tray seedlings remained robust at a level of 4.8-4.9 mm, while the stem thickness of the 72 hole tray seedlings decreased to 4.45 mm (a decrease of about 7.3%). The number of leaves in each treatment group remained stable (4.2-4.3 pieces) and did not show significant differences. Figure 7 compares the effects of different seedling tray specifications on the morphological indicators of underground growth of zucchini seedlings.



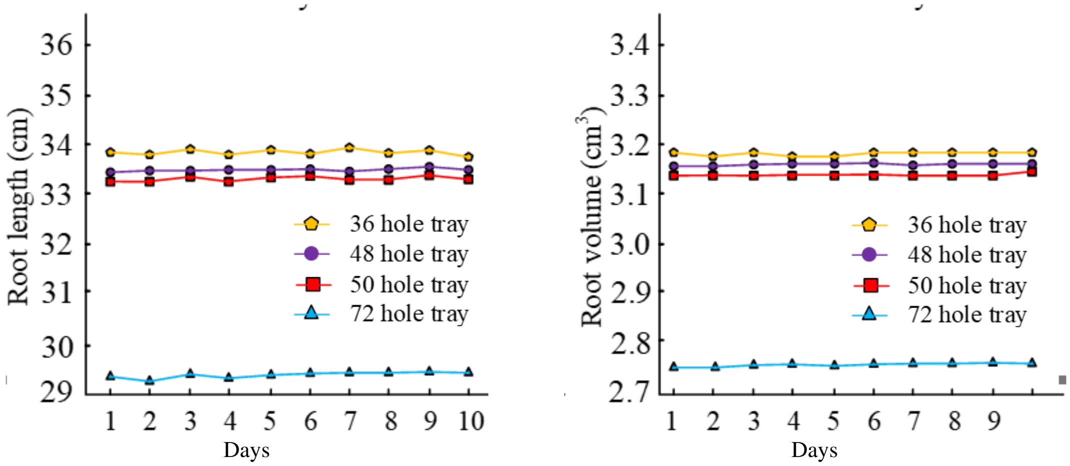
(a) (b)



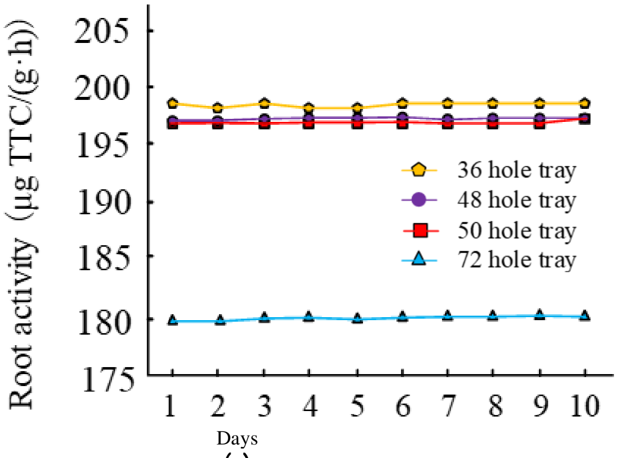
(c)

Figure 6. Effects of different seedling tray specifications (36-, 48-, 50-, and 72-hole trays) on above-ground morphological indicators of zucchini seedlings: (a) plant height, (b) stem thickness, and (c) number of leaves across different growth days

In Figure 7 (a), the root length of zucchini seedlings cultivated with 36 hole, 48 hole, and 50 hole trays remained stable at 33 cm-34 cm, while when 72 well trays were used, the plant height significantly decreased to 28.3 cm. The root volume index showed a similar trend. In Figure 7 (b), the root volume of the first three types of tray seedlings remained robust at a level of 3.1 cm^3-3.2 cm^3, while the root volume of the 72 hole tray seedlings decreased to 2.75 cm^3. In Figure 7 (c), the root activity of zucchini seedlings cultivated using the first three types of tray remained in the range of 195 μg TTC/(g·h) to 200 μg TTC/(g·h), while the root activity of 72 hole tray seedlings directly decreased to around 180 μg TTC/(g·h) (a decrease of about 8.3%). Table 3 compares the effects of different seedling tray specifications on the dry matter accumulation and water use of zucchini seedlings.



(a) (b)



(c)

Figure 7. Effects of different seedling tray specifications (36-, 48-, 50-, and 72-hole trays) on underground morphological indicators of zucchini seedlings: (a) root length, (b) root volume, and (c) root vitality across different growth days

Table 3. Effects of tray specifications (36-, 48-, 50-, and 72-hole) on water use efficiency (WUE), water consumption, and dry matter accumulation of zucchini seedlings

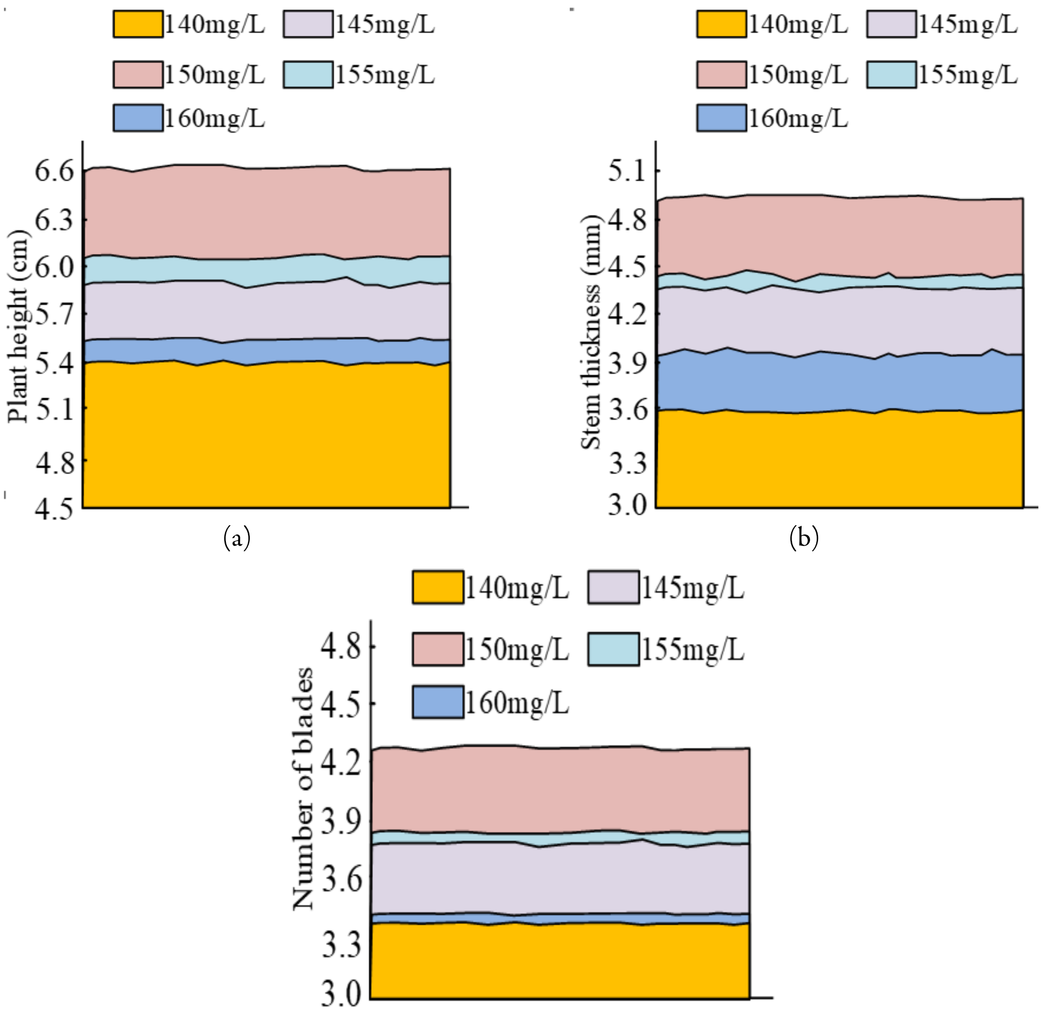
Tray specification	WUE (%)	Water consumption (mL)	Dry matter accumulation (g)
36-hole	52.38 ± 0.79 ^{a}	415.31 ± 0.98 ^{a}	3.80 ± 0.01 ^{a}
48-hole	52.68 ± 0.54 ^{a}	415.16 ± 0.81 ^{a}	3.76 ± 0.02 ^{a}
50-hole	51.60 ± 0.37 ^{a}	411.95 ± 1.54 ^{a}	3.79 ± 0.02 ^{a}
72-hole	44.68 ± 0.59 ^{b}	386.84 ± 0.47 ^{b}	3.07 ± 0.02 ^{b}

Mean ± standard deviation (n = 3). Within each column, means followed by the same letter are not significantly different, while means followed by different letters differ significantly ($p < 0.05$, Duncan's test)

Tray specification had a significant effect on the water use efficiency (WUE), water consumption, and dry matter accumulation of zucchini seedlings ($p < 0.05$). According to Duncan's multiple range test, seedlings grown in 36-, 48-, and 50-hole trays did not differ significantly from each other in any of the three parameters, maintaining high WUE values (51.60-52.68%), stable water consumption (411.95-415.31 mL), and consistent dry matter accumulation (3.76-3.80 g). In contrast, seedlings cultivated in 72-hole trays showed significantly lower performance across all indicators, with WUE decreasing to 44.68% (a reduction of approximately 14.7%), water consumption declining to 386.84 mL (about 6.2% lower), and dry matter accumulation dropping to 3.07 g (about 19.6% lower). These results indicate that tray designs with more than 50 holes impose ecological limitations by reducing the volume of each cultivation unit, thereby hindering root development and disrupting substrate moisture balance. Under these conditions, seedlings exhibited a stress response characterized by reduced transpiration, ultimately leading to a "low water consumption-low efficiency-low accumulation" growth pattern.

Effect of ACA concentration on the growth and WUE of zucchini seedlings

Figure 8 compares the effects of different concentrations of ACA on some morphological indicators of ground growth in zucchini seedlings.

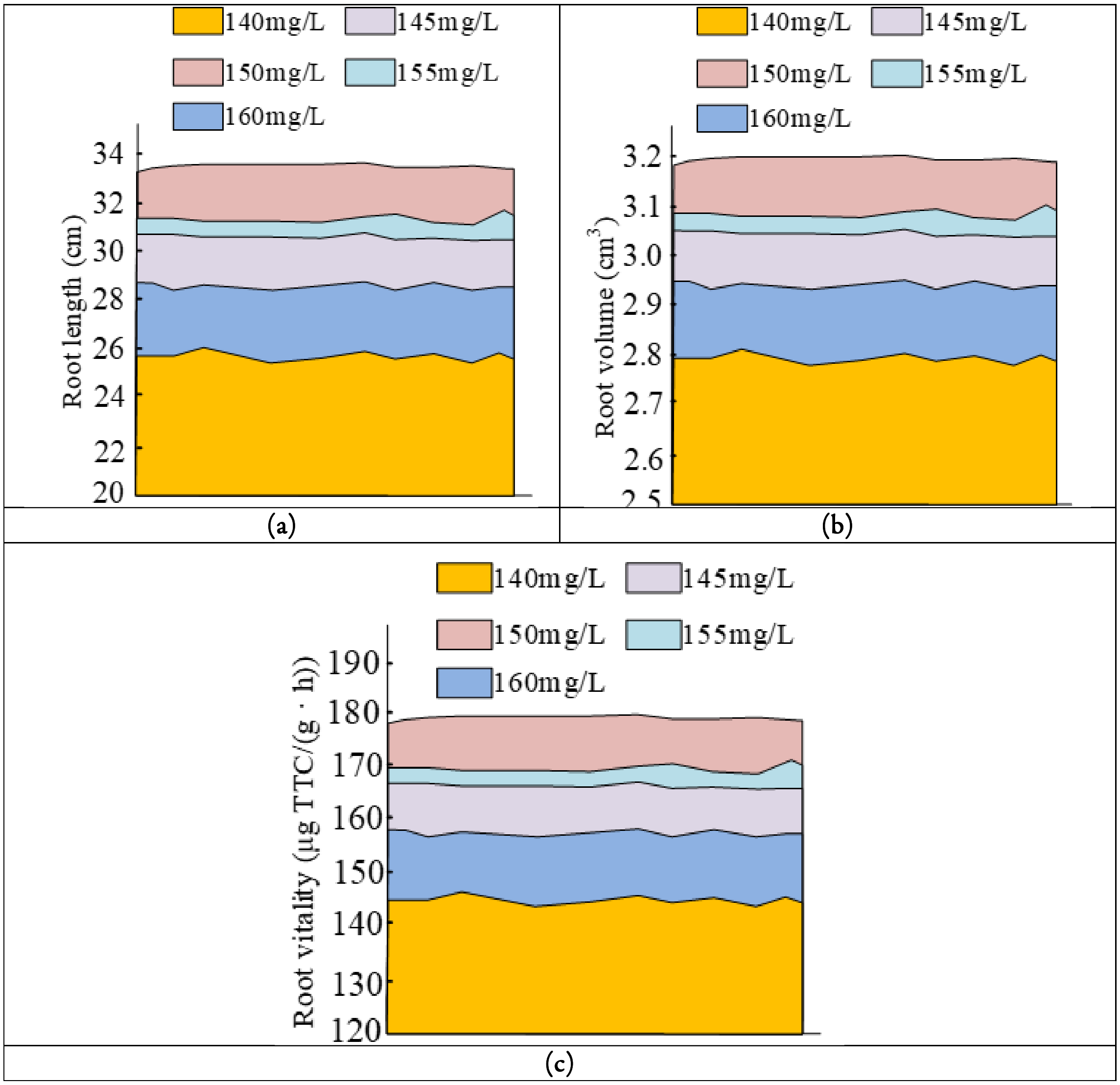


(a) (b)

(c)

Figure 8. Effects of ACA concentrations (140-160 mg L^{-1}) on the above-ground morphological traits of zucchini seedlings: (a) plant height, (b) stem thickness, and (c) number of blades. Data are presented as mean values with standard deviation bars

In Figure 8 (a), the height of zucchini seedlings was highest in the 150 mg L^{-1} ACA treatment, at approximately 6.61 cm, followed by ACA treatments at concentrations of 155 mg L^{-1}, 145 mg L^{-1}, 160 mg L^{-1}, and 140 mg L^{-1}. The treatment with 140 mg L^{-1} ACA resulted in the lowest plant height of approximately 5.40 cm ($p < 0.05$). The height of zucchini seedlings treated with 150 mg L^{-1} ACA increased by about 22.4% compared to the 140 mg L^{-1} treatment. In Figure 8 (b), the stem thickness of zucchini seedlings was highest in the 150 mg L^{-1} ACA treatment, at approximately 4.83 mm, followed by ACA treatments at concentrations of 155 mg L^{-1}, 145 mg L^{-1}, 160 mg L^{-1}, and 140 mg L^{-1}. The treatment with 140 mg L^{-1} ACA resulted in the lowest stem thickness of approximately 3.61 mm. The stem thickness of zucchini seedlings treated with 150 mg L^{-1} ACA increased by approximately 33.8% compared to the 140 mg L^{-1} treatment. In Figure 8 (c), the number of leaves in zucchini seedlings treated with 150 mg L^{-1} ACA was the highest, about 4.22, while the lowest number was 3.39 after 140 mg L^{-1} ACA treatment. Figure 9 compared the effects of different concentrations of ACA on the morphological indicators of underground growth in zucchini seedlings.



(a) (b)

(c)

Figure 9. Effects of ACA concentrations (140-160 mg L^{-1}) on the underground morphological traits of zucchini seedlings: (a) root length, (b) root volume, and (c) root vitality. Data are presented as mean values with standard deviation bars

In Figure 9 (a), the root length of zucchini seedlings was highest in the 150 mg L^{-1} ACA treatment, at approximately 33.2 cm, followed by ACA treatments at concentrations of 155 mg L^{-1}, 145 mg L^{-1}, 160 mg L^{-1}, and 140 mg L^{-1}. The ACA treatment at 140 mg L^{-1} resulted in the lowest root length of approximately 25.8 cm, with significant differences. In Figure 9 (b), the root volume of zucchini seedlings was highest in the 150 mg L^{-1} ACA treatment, at approximately 3.20 cm^3, followed by ACA treatments at concentrations of 155 mg L^{-1}, 145 mg L^{-1}, 160 mg L^{-1}, and 140 mg L^{-1}. The ACA treatment at 140 mg L^{-1} resulted in the lowest root volume of approximately 2.81 cm^3. In Figure 9 (c), the root activity of zucchini seedlings treated with 150 mg L^{-1} ACA was the highest, at approximately 179.23 TTC/(g·h). The root activity was the lowest after treatment with 140 mg L^{-1} ACA, at approximately 145.32 TTC/(g·h). Table 4 compared the WUE, water consumption, and dry matter accumulation of zucchini seedlings with different seedling tray specifications.

Table 4. Effects of ACA concentrations (140-160 mg L^{-1}) on water use efficiency (WUE), water consumption, and dry matter accumulation of zucchini seedlings. Data are presented as mean ± standard deviation (n = 3)

ACA of different concentrations	WUE (%)	Water consumption (mL)	Dry matter accumulation (g)
140 mg L^{-1}	30.15 ± 1.29 ^{c}	342.14 ± 6.98 ^{c}	2.75 ± 0.06 ^{c}
145 mg L^{-1}	37.14 ± 1.28 ^{b}	372.69 ± 6.65 ^{b}	2.92 ± 0.05 ^{b}
150 mg L^{-1}	44.33 ± 1.33 ^{a}	382.36 ± 6.35 ^{a}	3.32 ± 0.07 ^{a}
155 mg L^{-1}	39.63 ± 1.28 ^{b}	369.54 ± 6.48 ^{b}	3.05 ± 0.05 ^{b}
160 mg L^{-1}	34.33 ± 1.28 ^{bc}	35.42 ± 6.35 ^{bc}	2.85 ± 0.07 ^{bc}

Different letters within the same column indicate significant differences among treatments according to Duncan's multiple range test at $p < 0.05$

In Table 4, there are significant differences in WUE, water consumption, and dry matter accumulation of zucchini seedlings under different concentrations of ACA. Among them, the seedlings of zucchini treated with 150 mg L^{-1} ACA had the highest WUE, reaching 44.33% ± 1.33%, water consumption of 382.36 ± 6.35 mL, and dry matter accumulation of 3.32 ± 0.07 g, all of which were the highest. This indicated that a concentration of 150 mg L^{-1} ACA could effectively promote the WUE by zucchini seedlings and help them accumulate more dry matter, thereby promoting their growth. The seedlings of zucchini treated with 140 L^{-1} ACA had the lowest WUE (30.15% ± 1.29%), water consumption (342.14 ± 6.98 mL), and dry matter accumulation (2.75 ± 0.06 g). The WUE of zucchini seedlings treated with 150 L^{-1} ACA increased by about 46.9% compared to 140 L^{-1}. This is due to the low concentration of ACA, which cannot fully exert its promoting effect on seedling growth, resulting in limited seedling growth.

Discussion

A series of experiments were designed to explore the optimal conditions affecting the growth and development of zucchini seedlings. The substrate treatment method of grass charcoal composed by perlite: vermiculite=0.7:0.2:0.1 showed the best performance in all growth and water use efficiency (WUE) indicators. Compared with A (pure peat), the plant height, the stem thickness, the dry matter accumulation, and the WUE increased by 17.0%, 41.9%, 53.2%, and 40.6%, respectively. This result was highly consistent with the "matrix three-phase equilibrium model" proposed by Shalchian *et al.* (2023). The coarse pores of perlite maintained the oxygen diffusion rate at > 6 mg L^{-1}, significantly higher than the < 3 mg L^{-1} of pure peat. Vermiculite provided K^+, Mg^{2+}, and buffering through cation exchange, alleviating the nutrient deficiency of perlite. Further statistical tests showed that the D ratio was significantly better than the E ratio in terms of root length, root volume, and root activity (0.7:0.3:0). This indicated that when the perlite ratio exceeded 20%, the excessive pore size led to a decrease in substrate water holding capacity from 42% to 31%, a decrease in transpiration rate, thus, a subsequent decline in WUE. This result was consistent with the "water conservation strategy" proposed by Rivier *et al.* (2022) for seedling initiation.

There were no significant differences ($p > 0.05$) in plant height, stem thickness, dry matter accumulation, and WUE among seedlings in trays with 36, 48, and 50 holes. However, the 72 hole tray caused a sharp decrease of 14.7% and 19.6% in WUE and dry matter accumulation, respectively. This was consistent with the "small container effect" proposed by Ogasa *et al.* (2021). When the single pore volume decreased from 97.8 mL (36 pores) to 46.8 mL (72 pores), root domain limitation led to an increase in the average root diameter and a decrease in root hair density, resulting in a 14.3% reduction in absorption area. This triggered an up-regulation of ABA signal, a decrease in stomatal conductance, and limited transpiration, forming a trade-off pattern of "low water consumption - low growth". Although water consumption decreased, the decrease in dry matter was greater, resulting in a significant reduction in WUE. This result was consistent with the photosynthesis transpiration coupling model proposed by Han *et al.* (2023) under water stress.

Different concentrations of Alginate composite agent (ACA) had significant effects on the growth and WUE of zucchini seedlings. The treatment with 150 mg L^{-1} ACA increased WUE to 44.33%, which was 46.9% higher than the treatment with 140 mg L^{-1}. Wang *et al.* (2024 b) found that exogenous ASA cleared ROS through the ASA GSH cycle, maintained plasma membrane H^+- ATPase activity, promoted the opening of K^+ channels driven by root proton pumps, thereby increasing root pressure and water absorption (Wang *et al.* 2024 b)., In this study, treatment with 150 mg L^{-1} resulted in a root activity of 179 TTC/(g·h), an increase of 23.3% compared to 140 mg L^{-1}. However, under the treatment with 160 mg L^{-1}, WUE decreased to 34.33%, showing an "inverted U-shaped" dose-response, which was consistent with the Hormesis effect curve. High concentrations of ASA might induce excessive production of ROS (especially H_2O_2), trigger phosphorylation of stomatal closing protein OST1, and reduce stomatal conductance, thereby inhibiting photosynthesis transpiration coupling (Prodhan *et al.*, 2018).

Conclusion

The "optimal combination" for industrialized seedling cultivation of zucchini can be summarized as follows: substrate D ratio (peat: perlite: vermiculite = 0.7:0.2:0.1) + 50 hole tray + 150 mg L^{-1} ACA. This combination maintains a high individual plant resource space while balancing aeration, nutrient retention, and redox homeostasis, achieving a virtuous cycle of "high water consumption, high efficiency, and high accumulation. However, in actual seedling production, it is also necessary to select suitable seedling substrates, seedling tray specifications, and Alginate composite agent (ACA) concentrations based on specific needs and resource conditions to improve the growth quality and water use efficiency (WUE) of zucchini seedlings.

Authors' Contributions

Conceptualization: LP, KY, YZL; Methodology: YZL, FZ; Validation: YZL, LLC; Formal analysis: GX, LP; Investigation: KY, LLC; Resources: YZL, LP; Writing - original draft: LP, KY, FZ; Writing - review and editing: YZL, LLC; Visualization: GX, KY; Supervision: LP; Project administration: YZL.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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