

# Effects of plug tray cell size on the growth of *Atractylodes Chinensis* (DC) Koidz seedlings

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**Abstract:** *Atractylodes Chinensis* (DC) Koidz is a perennial herb often used as a prescription medicine for influenza, to invigorate the spleen and remove dampness. The quality of the herb is determined by the quality of the seedlings. The traditional *A. chinensis* seedling production technique has nonuniform seedlings and low mechanisation. The plug seedling technique was used to produce *A. Chinensis* seedlings. This study was conducted to determine the growth characteristics of *A. Chinensis* seedlings according to the cell size of plug trays and number of days after sowing. Plant height, stem diameter, rhizome diameter, number of leaves, leaf area, and shoot dry and fresh weight of *A. Chinensis* seedlings were significantly higher in the P32-D treatment than in the P72, P50, and P32 treatments, but not significantly different from the P28-D treatment 60 d after sowing. The P28-D treatment resulted in a considerable drop in rhizome fresh weight and healthy seedling index, both of which are key indicators for assessing seedling quality. Although the differences in Pn among treatments were not significantly different, Tr was considerably greater in *A. Chinensis* seedlings treated with P32-D and P28-D than in the other three treatments. The P32-D and P28-D treatments had considerably greater potential maximum photochemical efficiency of PSII, quantum yield of photosystem II, photochemical quenching and electron transport rate than the other three treatments. Root vitality of *A. Chinensis* seedlings was significantly stronger in the P32-D treatment than in the other four treatments, and it was 1.9 times higher than in the P28-D treatment. The soluble protein, soluble sugar, and starch contents of *A. Chinensis* seedlings were highest in the P32-D treatment, but the differences among treatments were not significant. LUE, EUE, PY and EY were significantly higher in the P32-D treatment than in the other four treatments, being 1.3, 1.3, 1.4 and 1.4 times higher than in P28-D, respectively. On the other hand, as for the change in the growth of *A. Chinensis* seedlings according to the number of days after sowing, the growth of shoots and rhizome was most vigorous at the 60 d after sowing, while the fresh weight of the rhizome, which can be considered as an indicator of root growth, increased steadily during the experiment but slowed down after 60 d. As a result, 32-cell deepened plug trays and a seedling nursery for a period of 60-75 d are recommended for commercial cultivation of *A. Chinensis* seedlings. This will provide technical support for production in *A. Chinensis* seedling.

**Keywords:** *Atractylodes Chinensis* (DC) Koidz, plug seedling, plug tray cell size

**DOI:** 10.25165/j.ijabe.20241704.8916

**Citation:** Shi K X, Liang J W, Ji F, He D X. Effects of plug tray cell size on the growth of *Atractylodes Chinensis* (DC) Koidz seedlings. Int J Agric & Biol Eng, 2024; 17(4): 45–52.

## 1 Introduction

*Atractylodes* is a genus of plants in the *Asteraceae* family, a perennial herb<sup>[1]</sup>. *Atractylodes* is widespread in China, Korea, Japan and other countries. *Atractylodes Chinensis* (DC) Koidz is a Chinese variety known as BeiCangzhu. *Atractylodes* is widely used in a variety of crude medicines prescribed, has the effect of invigorating the spleen and eliminating dampness<sup>[2,3]</sup>. *Atractylodes* has traditionally been used as an important herbal medicine for the treatment of influenza for a long time. *Atractylodes* is listed in the 2019 Classification of Infectious Diseases (COVID-19) and ranks

first among the key Chinese herbal medicines for national epidemic prevention and control in China<sup>[4]</sup>.

In recent years, the amount of wild *Atractylodes* has decreased, making it difficult to meet market demand<sup>[5]</sup>. The area under cultivation of *Atractylodes* has increased. In production, *Atractylodes* is mainly grown by seedling transplantation. The quality of the seedling determines its survival rate after transplanting as well as the quality of the herb. Unlike traditional horticultural crops, seedling standards for medicinal herbs are specific. In Hebei Province, China, *Atractylodes Chinensis* seedlings must meet the following standards<sup>[6]</sup>: plant height at least 5 cm, number of leaves at least 4, stem diameter at least 0.2 cm. In addition, in production, a rhizome diameter of more than 1cm and a rhizome fresh weight of more than 1.5 g are also used as evaluation standards for *Atractylodes Chinensis* seedlings<sup>[7]</sup>. Seedlings meeting these standards only can be transplanted into herb production fields, which often takes 1-2 years. Seedling transplantation relies mainly on manual labour and the degree of mechanisation is low. This is the main reason why large-scale production of *Atractylodes* is limited.

Plug seedling is an essential horticultural production method

**Received date:** 2024-03-11 **Accepted date:** 2024-07-18

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that is widely used in vegetable production<sup>[8]</sup>. Plug production is the planned production of healthy and standardised seedlings throughout the year<sup>[9]</sup>. Plug production can be combined with machinery to increase productivity<sup>[10]</sup>. Plug seedling transplanting causes little damage to the plant root system, short seedling recovery time and high seedling survival rate<sup>[11]</sup>. In addition, plug seedling saves seed and reduces production costs<sup>[8]</sup>. In recent years, there has been an increasing number of studies on plug seedling of medicinal plants. According to Jeong et al.<sup>[12]</sup>, utilizing 128-cell plug trays with 2 seeds per cell produced uniform and healthy *Astragalus membranaceus* seedlings. Liu<sup>[13]</sup> successfully cultivated three medicinal plants in plug trays: *Astragalus membranaceus*, *Adenophora triphylla*, and *Codonopsis lanceolata*. Lee et al.<sup>[14]</sup> designed a novel plug tray to maximize space utilization in greenhouses and plant factories while also optimizing ginseng seedling planting density. There is little research on plug seedling production of *Atractylodes* and further research is needed to provide support for seedling production.

Plug cell size is one of the most important factors affecting seedling growth. Appropriate plug cell size promotes root development and is beneficial to seedling growth. In general, larger cells result in higher early yields and are easier to manage because the increased soil volume holds more water and nutrients<sup>[15]</sup>. Žnidarčič et al.<sup>[16]</sup> found that increasing cell volume increased leaf height, leaf number and fresh leaf mass in maize salad. A trend among many commercial vegetable growers is to increase the number of cells per tray (smaller cells) so that more plants can be grown in the limited space available. Park et al.<sup>[17]</sup> evaluated vegetables of the *Compositae* and *Cruciferae* families grown in 50, 72, 128 and 162 cell plug trays and found that 162 and 72 cell plug trays significantly improved the yield and quality of *Compositae* and *Cruciferae* vegetables, respectively. Oh et al.<sup>[18]</sup> investigated the effect of 72, 128 and 200 cell plug trays on the growth of *Veronica pusanensis* and found that there was no difference in the growth of seedlings according to the size of the plug trays, but the production of seedlings in the 200-cell plug trays appeared to be economically advantageous. Park et al.<sup>[19]</sup> investigated the effect of four types of plug trays (143, 70, 18, 13 mL/cell) on the growth of *Hemerocallis thunbergii* Baker seedlings and recommended the selection of 18 mL/cell plug trays, taking into account the production cycle and economic efficiency. Economic efficiency is also very important in determining whether or not a seedling production method can be commercialised. In this study, light energy use efficiency (LUE), electrical energy use efficiency (EUE), photon yield (PY) and energy yield (EY) were used as indicators to evaluate the energy use efficiency of seedling production<sup>[20]</sup>. The size of the plug cell has a variable effect on various plants. Herb, which is used as root or rhizome for medicinal purposes, has a different need for a plug tray than horticultural crops such as vegetables. It is vital to choose a plug tray size that is both suitable and economical for seedling growth. Therefore, plug cell size is a very important part of research on plug seedling of medicinal plants.

There are differences between the artificial cultivation environment and the wild natural growth environment of medicinal plants. Some of the medicinal plants under the forest are placed in the open field for intensive cultivation, which leads to the inferior composition of medicinal plants<sup>[21]</sup>. Light affects the growth and development of medicinal plants, physiological and biochemical characteristics, but also affects the accumulation of plant secondary metabolites. Guo et al.<sup>[22]</sup> found that mild shading promotes the synthesis and accumulation of sesquiterpenoids in *Atractylodes*

*lancea* rhizome by regulating photosynthetic efficiency and phytohormone production, thereby promoting transcriptional expression. Photosynthesis is the basis of plant growth and metabolism, and the photosynthesis of plants will change under different light conditions. The change in light will directly affect the change in chloroplast structure and activity in plant leaves, for example, the content of chlorophyll *b* will increase under low light. At the same time, low light will affect other physiological processes in plants, thereby indirectly affecting photosynthesis, such as lower stomatal conductance, lower transpiration rate, lower water use efficiency, and blocked transport of photosynthetic products, resulting in accumulation of photosynthetic products starch and sucrose in leaves. The quality formation of medicinal plants is directly or indirectly influenced by the physiological state of plants. In general, plants with better physiological qualities-such as enhanced stress tolerance and more effective photosynthesis are able to create higher-quality products than plants with weaker physiological traits<sup>[21]</sup>. Therefore, photosynthetic and chlorophyll fluorescence parameters are also used in this paper as an indicator to support the evaluation of seedling quality<sup>[23]</sup>.

In order to further investigate the viability of plug seedlings for *Atractylodes Chinensis* seedlings. This study investigated the growth characteristics, photosynthetic characteristics, chlorophyll fluorescence characteristics, physiological and biochemical characteristics of rhizomes, and energy use efficiency of *Atractylodes Chinensis* seedlings based on the size of plug tray and number of days after sowing. Finally, the appropriate size of plug tray and number of days of seedling cultivation were determined.

## 2 Materials and methods

### 2.1 Plant materials

The seeds of *Atractylodes Chinensis* (DC) Koidz used in this study were harvested in October 2022 in the field (41.53°N, 117.25°E) of Longhua County, Chengde City, Hebei Province. The harvested seeds were used in this experiment after being dried at room temperature for a week and stored at 4°C with impurities removed. Seedlings were planted in plug trays with one seed per cell. Nutrient solution has been irrigated since the first leaf development. Japanese horticultural experimental nutrient solution was utilized with an electrical conductivity of 1.4-1.6 mS/cm and pH 6.0-6.5 and comprised the following components (mg/L): Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 944; KNO<sub>3</sub>, 808; MgSO<sub>4</sub>·7H<sub>2</sub>O, 492; NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 152; Na<sub>2</sub>Fe<sub>7</sub>-DTPA, 22.55; MnSO<sub>4</sub>·4H<sub>2</sub>O, 1.54; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.08; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.22; H<sub>3</sub>BO<sub>3</sub>, 2.86; (NH<sub>4</sub>)<sub>6</sub>Mo<sub>6</sub>O<sub>2</sub>·4H<sub>2</sub>O, 0.02<sup>[24]</sup>.

### 2.2 Growth conditions

The seedlings were cultivated in a plant factory (China Agricultural University, Beijing). The environment in the growth chamber was controlled as follows: 12 h/12 h light/dark photoperiod with a photosynthetic photon flux density of 250 μmol/(m<sup>2</sup>·s) (W-LED-18W, 300-800 nm, R:B=1.8 and R:FR=8.9, Beijing Lighting Valley Technology Co., Ltd., China); air temperature in the light and dark periods was (23±1)°C and (18±1)°C, respectively; relative humidity was (60±10)%; CO<sub>2</sub> concentration was (800±50) μmol/mol in the light period and (400±50) μmol/mol in the darkness.

### 2.3 Plug treatments

To investigate the growth of the seedlings based on the size of plug trays, in March 2023, horticultural substrate (peat: vermiculite: perlite volume ratio of 1:1:1) was filled into regular and deepened plug trays. The sizes of regular plug trays (Figures 1a-1c) are as follows: 72 cells (W 280×L 540×H 50, mm; 38 mL/cell), 50 cells (W 280×L 540×H 50, mm; 55 mL/cell), and 32 cells (W 280×

L 540×H 50, mm; 100 mL/cell). The sizes of deepened plug trays (Figures 1d-e) are as follows: 32 cells (W 280×L 540×H 110, mm; 190 mL/cell) and 28 cells (W 280×L 540×H 150, mm; 390 mL/cell).

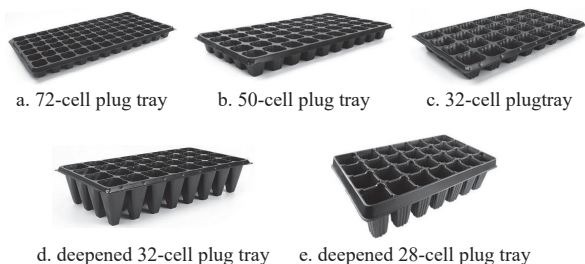


Figure 1 Plug trays of different experimental treatments

## 2.4 Growth measurements

### 2.4.1 Growth characteristics

The experiment was conducted 60 d after sowing and 8 plants were randomly selected from each treatment. For survey items, plant height, stem diameter, rhizome diameter, leaf numbers, leaf area, fresh weight, shoot and rhizome of dry weight, and shoot and rhizome of fresh weight were measured. The whole seedlings were oven-dried at 105°C for 3 h and subsequently set to 80°C for over 72 h for measuring the dry weights. Leaf area of seedlings was measured by a scanner [LiDE110, Canon (China) Co., Ltd., China] and calculated by image processing. The healthy seedling index (HI) was calculated from the above data using the following formula<sup>[25]</sup>:

$$HI = \left( \frac{D_s}{D_r} + \frac{DW_R}{DW_S} \right) \times DW_T \quad (1)$$

where,  $D_s$  is the diameter of stem, mm;  $D_r$  is the diameter of rhizome, mm;  $DW_R$  is the dry weight of the rhizome, g;  $DW_S$  is the dry weight of the shoot, g;  $DW_T$  is the dry weight of the total plant, g.

The stem diameter in this formula measures the diameter of the aboveground base of the plant.

### 2.4.2 Photosynthetic parameters

Photosynthetic parameters were measured at 60 d after sowing. A portable photosynthesis system (LI-6400XT, LI-COR Inc., Lincoln, USA) was used to measure the photosynthetic parameters including net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Cond), intercellular CO<sub>2</sub> concentration (Ci). In the leaf chamber, light intensity, leaf chamber temperature, air velocity, and CO<sub>2</sub> concentration were controlled at 250 μmol/(m<sup>2</sup>·s), 23°C, 500 μmol/s, and 800 μmol/mol, respectively.

### 2.4.3 Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were measured at 60 days after sowing. The potential maximum photochemical efficiency of PSII (Fv/Fm), the quantum yield of photosystem II (ΦPSII), photochemical quenching (qP) and the electron transport rate (ETR) were calculated by a dual channel fluorometer (Dual-PAM-100, WALZ company, Germany). Before measurement, the sample leaves were put in darkness for 30 min.

### 2.4.4 Physiological and biochemical parameters

In this study, root vitality was measured by TTC (2,3,5-triphenyltetrazolium chloride) method<sup>[26]</sup>, soluble protein content by Bradford method<sup>[27]</sup>, soluble sugar content by anthrone colorimetric methods and starch content by anthrone sulphuric acid method<sup>[28]</sup>.

### 2.4.5 Energy use efficiency

Light energy use efficiency (LUE), electric energy use efficiency (EUE), photon yield (PY) and energy yield (EY) were

calculated to evaluate the energy use efficiency of different treatments. The formula are as follows<sup>[29,30]</sup>:

$$LUE = f \times D / PAR \quad (2)$$

$$EUE = f \times D / W \quad (3)$$

$$PY = FW / OTLI \quad (4)$$

$$EY = FW / OPLI \quad (5)$$

where,  $f$  is the chemical energy of the plant's dry matter, 20 MJ/kg;  $D$  is the increase in the dry weight of the available part of the plant, kg/m<sup>2</sup>; PAR is the photosynthetically active radiation received by the plant between 300-800 nm, MJ/m<sup>2</sup>;  $W$  is the electrical energy consumed by the LEDs, MJ/m<sup>2</sup>; FW is the fresh weight of the marketable part of a plant, g; OTLI is the average number of light quantum received at 15 cm under the lamps during growth per plant, mol; OPLI is the average energy consumption of lighting by a single plant during growth per plant, kW·h.

## 2.5 Statistical analysis

IBM SPSS Statistics 26 (IBM Corporation, Chicago, IL, USA) was used for statistical analysis. The comparisons were conducted by Duncan's multiple range test selected from significant one-way ANOVA ( $p < 0.05$ ). The results were recorded as mean ± standard deviation values ( $n=8$ ).

## 3 Results and discussion

### 3.1 Growth characteristics of *A. Chinensis* seedlings

There were significant differences in plant height, stem diameter, rhizome diameter, leaf numbers, leaf area, fresh weight, shoot and rhizome of dry weight, and shoot and rhizome of fresh weight of *A. Chinensis* seedlings in different treatments (Table 1 and Figure 2). Treatment P32-D had a significantly greater plant height, stem diameter, and rhizome diameter than treatments P72, P50, and P32, but not statistically different from treatment P28-D. Leaf area of P32-D and P28-D treatments was significantly larger than the other three treatments, but the difference between them was not significant. Dry and fresh weights of shoots and rhizomes of P32-D and P28-D treatments were greater than those of the other three treatments. Deepened plug trays (P32-D and P28-D) produced seedlings with more strong root development than regular plug trays (Figure 2). During ginseng seedling culture, Lee et al.<sup>[12]</sup> discovered that increasing plug tray depth boosted root dry and fresh weight, root diameter, and leaf area of ginseng seedlings. Increased nutrient intake results from vigorous root development, which supports the growth of above-ground components<sup>[31]</sup>. Deepened plug treatments produced more leaf area and biomass accumulation than regular plug treatments in this study. The P28-D treated seedlings had longer roots than the P32-D treatment, but their rhizomes showed a considerable drop in diameter and fresh weight. Plant growth is widely recognized to have both promoting and competitive interactions<sup>[32]</sup>. This occurs as well in the development of roots and rhizomes. Park et al.<sup>[17]</sup> discovered that 50 cell plug trays demonstrated no significant change from 32 cell plug trays, and that the leaf length was somewhat greater when compared to 32 cell plug trays. This is consistent with the findings of our research. The development of rhizomes is vital as we generate commodities. As a result, increasing the plug depth to stimulate root development while maintaining the optimum plug size for growth is more essential.

The greatest HI was P32-D, followed by P28-D and P32, while

the lowest was P72. The HI of P32-D treatment was 1.6 and 3.1 times higher than that of P72 and P28-D, respectively. The HI was composed of plant height, stem diameter, shoot dry weight, rhizome dry weight, and total plant dry weight. The plugs with large volume have improved these indexes (seedling height, stem diameter, shoot dry weight, and rhizome dry weight) whereas exceedingly small volume plugs have significantly restricted the growth of the *A. Chinensis* seedlings. The results of this paper showed that the HI of

P28-D treatment decreased significantly at 60 d after sowing. The reason may be that the root system needs to grow to a certain length to absorb water through the bottom of the plug tray, resulting in a slower rate of seedling growth in the early stage. Zheng et al.<sup>[25]</sup> found that the HI first increased and then decreased as the plug volume was reduced in the growth of *Miscanthus* seedlings. Root development is affected by the depth of the plug tray.

**Table 1 Growth of *A. Chinensis* seedlings measured at 60 d after sowing as affected by plug cell size**

Treatment	Height/cm	Stem diameter/mm	Rhizome diameter/cm	Leaf		Fresh weight/g		Dry weight/g		HI
				Number	Area/cm <sup>2</sup>	Shoot	Rhizome	Shoot	Rhizome	
P72	5.7±0.5c	2.1±0.2b	0.8±0.1c	4±1b	18.7±2.8c	0.6±0.1c	0.4±0.1c	0.2±0.0c	0.2±0.0c	0.7±0.1d
P50	6.3±0.7bc	2.5±0.2b	1.2±0.1b	4±1b	26.7±4.8bc	0.8±0.1b	0.7±0.1b	0.4±0.1b	0.3±0.1b	1.2±0.2c
P32	6.8±0.4b	2.7±0.3ab	1.2±0.1b	5±1ab	29.8±5.2b	0.9±0.1b	0.8±0.1b	0.4±0.0b	0.4±0.1b	1.5±0.2b
P32-D	8.0±0.6a	3.8±0.3a	1.6±0.1a	5±1ab	50.8±6.6a	1.5±0.3a	1.1±0.3a	0.6±0.1a	0.5±0.1a	2.2±0.2a
28-D	8.0±0.3a	3.2±0.2a	1.5±0.0a	6±1a	50.1±5.6a	1.6±0.1a	0.8±0.1b	0.7±0.2a	0.4±0.1b	1.3±0.3bc

Note: Different letters in the same column indicate significant differences at  $p=0.05$  level ( $n=8$ ) according to Duncan's test.



Figure 2 Effect of plug trays on the morphology of *A. Chinensis* seedlings after 15 d, 30 d, 45 d and 60 d cultivation of different experimental treatments

### 3.2 Photosynthesis and Chlorophyll fluorescence parameters of *A. Chinensis* seedlings

With P28-D being the highest treatment, P32-D coming in second, and P50 being the lowest, there were notable variances among the five Pn treatments (Table 2). This conclusion may be explained by the fact that Pn reflects the instantaneous photosynthetic rate of a single leaf, while photosynthesis in plants is also proportional to the number of leaves and leaf area<sup>[33]</sup>. Cond was highest for P28-D and lowest with P72, with no significant differences across the five treatments. Ci differed substantially across the five treatments, with P32-D having a much lower Ci than the other treatments, indicating more photosynthesis. In comparison to the other three treatments, P32-D and P28-D showed substantially greater Tr levels. In particular, Tr was 1.4 times greater in P32-D than in P72, 1.3 times higher in P50, and 1.1 times higher in P32. This may be due to the deeper plug tray providing more water to the seedling root system, thereby promoting leaf Cond and Pn. Sung et al.<sup>[34]</sup> found that leaf diffusive resistance increases as leaf water potential decreases, leading to stomatal closure. Stomata are important for the regulation of transpiration in

higher plants. Stomatal closure leads to a decrease in transpiration rate. Decreases in stomatal conductance and transpiration rate lead to decreases in photosynthetic rate.

There were significant differences in Fv/Fm,  $\Phi$ PSII, qP and ETR of *A. Chinensis* seedlings in different treatments (Figure 3). Fv/Fm reflects the potential maximum photosynthetic rate of plants and was mainly used to measure the potential activity of PS II in plant leaves<sup>[35]</sup>. Fv/Fm was highest in P32-D and lowest in P50, but the differences between treatments were not significant except for P50. The effective quantum yield of electron transport ( $\Phi$ PSII) is the actual photochemical efficiency of plant leaves when the PS II reaction center is closed under light. The  $\Phi$ PSII of P32-D and P28-D was significantly higher than the other three treatments, indicating stronger photosynthesis. The qP reflects the degree of opening of the PS II reaction center. The qP of P32-D and P28-D was significantly higher than the other three treatments. The ETR reflects the electron transfer rate of the PS II reaction center. The ETR of P32-D and P28-D was also significantly greater than the other three treatments. The results showed that Fv/Fm,  $\Phi$ PSII, qP and ETR of seedlings in deepened plug treatment were higher than



those in regular plug.

### 3.3 Physiological and biochemical of *A. Chinensis* seedlings

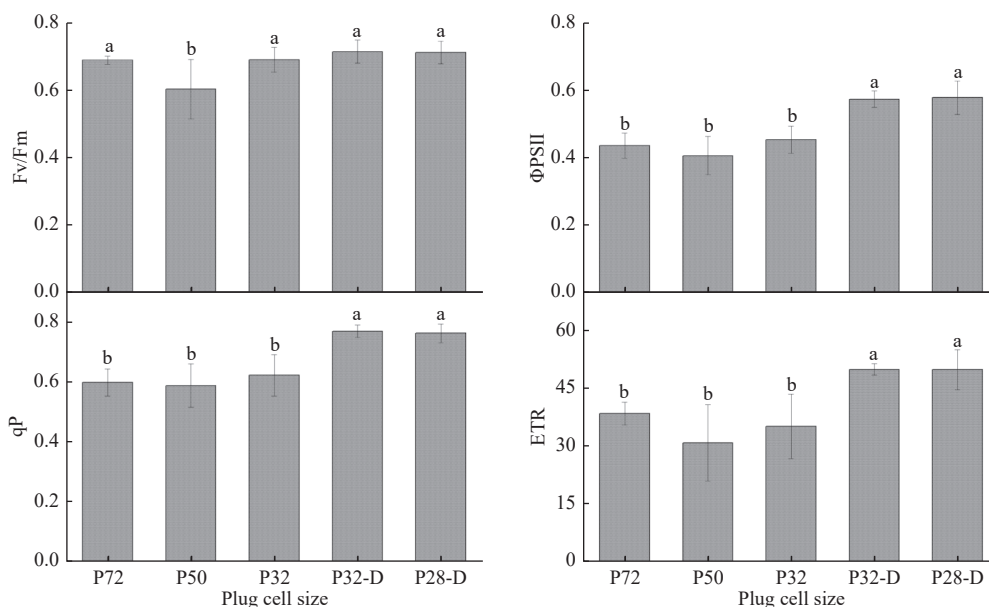
The P32-D treatment had the highest root vitality, which was 1.85 times greater than P28-D and significantly greater than the other four treatments (Table 3). This might be due to the low water content of the substrate as a result of the small size of the plug tray, which limits water uptake by the seedling roots. Zhou et al. found that drought causes a decrease in both root vitality and chlorophyll fluorescence parameters in strawberry seedlings, which is consistent

with this study<sup>[36]</sup>. The P32-D and P72 treatments had the highest soluble protein content of the rhizomes, but the difference between the five treatments was not significant. The soluble sugar content of the rhizomes was significantly higher in P32-D than in the other treatments, followed by P28-D, P72 and P32, and lowest in P50. The starch content was highest in P32-D and lowest in P28-D, with a 1.2-fold difference between the two treatments. The deeper plug tray may offer sufficient water and nutrients for seedling development, boosting seedling metabolite accumulation<sup>[37]</sup>.

**Table 2 Photosynthetic parameters of *A. Chinensis* seedlings under different plug tray cell size**

Treatment	Pn/ $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$			Cond/ $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$			Ci/ $\mu\text{mol}\cdot\text{mol}^{-1}$			Tr/ $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$						
P72	6.1	±	0.5	ab	115	±	8	ns	563	±	37	c	1.39	±	0.26	c
P50	6.1	±	0.7	b	116	±	15	ns	578	±	31	c	1.45	±	0.16	c
P32	6.6	±	0.3	ab	116	±	7	ns	605	±	22	d	1.79	±	0.18	b
P32-D	6.7	±	0.4	ab	117	±	14	ns	511	±	16	a	1.88	±	0.36	a
P28-D	6.9	±	0.4	a	119	±	20	ns	531	±	30	b	1.87	±	0.36	a

Note: Different letters in the same column indicate significant differences, NS represent no significant difference in the same column at  $p=0.05$  level ( $n=8$ ) according to Duncan's test.



Note: Different letters in the same column indicate significant differences at  $p=0.05$  level ( $n=8$ ) according to Duncan's test.

**Figure 3 Chlorophyll fluorescence of *A. Chinensis* seedlings under different plug tray cell size**

**Table 3 Physiological and biochemical properties of the rhizomes of *A. Chinensis* seedlings under different plug tray cell size**

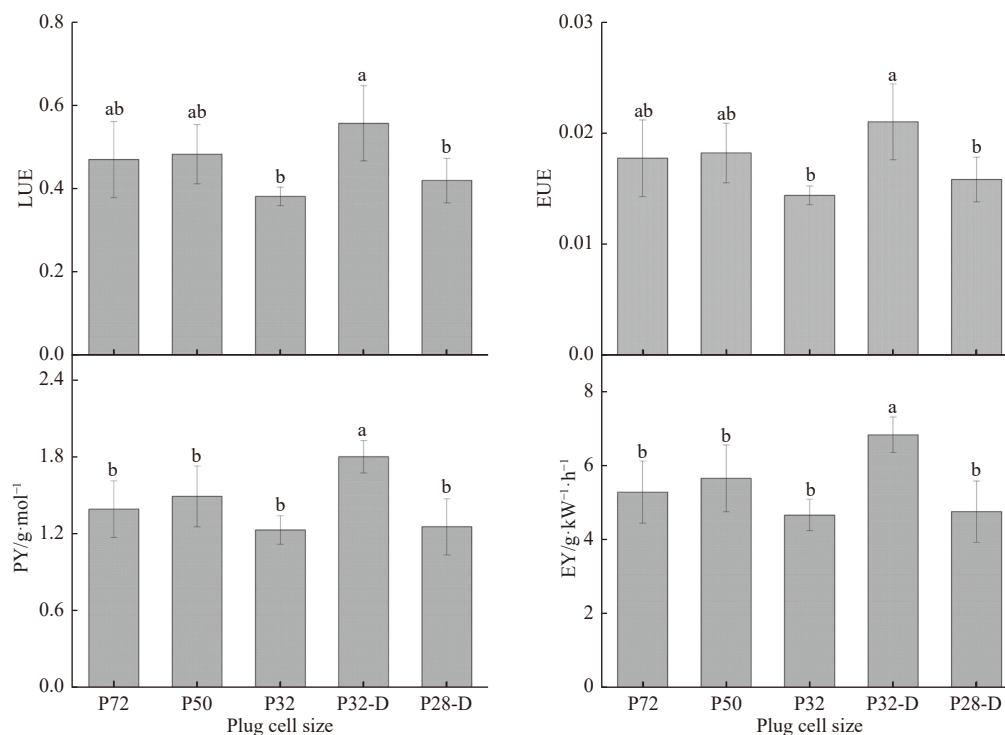
Treatment	Root vitality/ $\text{mg TTF}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$			Soluble Protein Content/ $\text{mg}\cdot\text{g}^{-1}$			Soluble Sugar Content/ $\text{mg}\cdot\text{g}^{-1}$			Starch Content/ $\text{mg}\cdot\text{g}^{-1}$						
P72	0.40	±	0.07	d	5.5	±	0.2	ns	209.6	±	18.8	ab	536.3	±	67.6	ns
P50	0.41	±	0.03	d	5.0	±	0.5	ns	176.8	±	35.6	b	512.4	±	65.3	ns
P32	0.67	±	0.09	b	5.2	±	0.9	ns	190.1	±	15.3	ab	494.3	±	55.5	ns
P32-D	1.04	±	0.11	a	5.5	±	0.5	ns	221.5	±	25.8	a	553.7	±	24.1	ns
P28-D	0.56	±	0.11	c	5.1	±	0.8	ns	209.8	±	37.7	ab	475.6	±	61.8	ns

Note: Different letters in the same column indicate significant differences, NS represent no significant difference in the same column at  $p=0.05$  level ( $n=8$ ) according to Duncan's test.

### 3.4 Energy use efficiency of *A. Chinensis* seedlings

Yokoi et al. and Kozai proposed that light energy use efficiency (LUE) and electric energy use efficiency (EUE) be measured using plant biomass accumulation. Plant dry matter accumulation has a close connection to the absorption and transformation of light energy<sup>[29,30]</sup>. Energy use efficiency was calculated for the different treatments and it was found that there was a consistent trend in LUE, EUE, PY and EY among the treatments (Figure 4). All showed that P32-D was the highest, followed by P50 and P72, and

P28-D and P32 were the lowest. There was a significant difference in LUE among the five treatments. P32-D had the highest LUE, which was 1.3 times higher compared to P28-D. Similarly, EUE of P32-D was 1.3 times higher than that of P28-D. PY of P32-D was the highest and significantly higher than that of the other four treatments. PY of P32-D was 1.4 times higher than that of P28-D. The results of EY were consistent with PY, which was significantly higher in P32-D than that of the other four treatments and was 1.4 times higher than that of P28-D. High energy consumption is a



Note: Different letters in the same column indicate significant differences at  $p=0.05$  level ( $n=8$ ) according to Duncan's test.

Figure 4 Efficiency of capacity utilization of *A. Chinensis* seedlings under different plug tray cell size

significant issue in seedling factory production. Improving energy efficiency and lowering costs are prerequisites for commercial production of *A. Chinensis* seedlings. As a result, energy use efficiency is an important factor to consider while selecting plug trays.

### 3.5 Cultivation period of *A. Chinensis* seedlings in 32-cell deepened plug trays

In addition, this study investigated the cultivation time of *A. Chinensis* seedlings using 32-cell deepened plug trays. It was found that the seedlings met the standards when the seedling period was 60 d (Table 4). Plant height of *A. Chinensis* increased significantly at 15 d and 30 d after sowing and slowed down at 45 d. The stem diameter of *A. Chinensis* changed very little at 15 d, 30 d and 45 d after sowing and became significantly larger at 60 d. At 60 d after sowing, the diameter of the rhizome was 1.6 cm, 1.6 times larger than at 45 d, and at 75 d and 90 d, it was only 1.1 and 1.2 times larger than at 60 d, respectively. The first leaf of *A. Chinensis* developed at 7 d after sowing and became an unfolding leaf at 15 d. At 30 d, 45 d, 60 d, 75 d and 90 d after sowing, the number of unfolding leaves was 3-4, 4-5, 5-7, 7-9 and 7-10, respectively (Figure 5). The number and area of unfolded leaves increased more slowly after 75 d. The growth of fresh weight of *A. Chinensis* overtime followed a sigmoid growth curve, with the fastest growth from 45 to 60 d after sowing and then gradually slowing down

(Figure 6). The difference in fresh weight of rhizomes at 60 d and 75 d after sowing was not significant. The fresh weight of the roots increased more at 75 d than at 60 d after sowing. The change in fresh weight at 90 d after sowing was similar to that at 75 d. The growth of dry weight of *A. Chinensis* with time followed a sigmoid growth curve, the fastest growth rate being observed from 45 to 60 d after sowing and then gradually slowing down. There was no significant difference in the dry weight of rhizomes, roots and leaves of *A. Chinensis* at 60 d and 75 d after sowing. Calculation of healthy seedling index of *A. Chinensis* showed that it increased with increasing cultivation time and was maximum at 90 d after sowing, while the difference in healthy seedling index at 75 d, 60 d after sowing was not significant. Park et al.<sup>[17]</sup> conducted research to determine the growth characteristics of HTB seedlings based on the number of days after sowing and the cell size of plug trays, and the results indicated that there is a certain correlation between the selection of plug trays and the number of days of seedling rearing, and a reasonable choice of the two to achieve economic maximization.

Improving energy use efficiency is the basis for commercialization. In order to investigate the seedling cultivation time suitable for factory production, the energy use efficiency at different times was calculated (Table 5). It was found that the energy use efficiency tended to stabilize at 75 d and 90 d after

Table 4 Growth of *A. Chinensis* seedlings measured at 15, 30, 45, 60, 75 and 90 d after sowing in 32-cell deepened plug trays

Day after sowing/d	Height/cm	Stem diameter/mm	Rhizome		Leaf		HI
			Diameter/cm	Fresh weight/g	Number	Area/cm <sup>2</sup>	
15	3.2±0.5c	2.2±0.2d	0.2±0.0e	0.1±0.0d	1±0d	1.3±0.3e	0.04±0.0e
30	6.9±0.8b	2.1±0.2d	0.5±0.0d	0.1±0.0d	3±0c	19.8±2.6d	0.13±0.0d
45	8.6±0.7a	2.3±0.1d	1.0±0.1c	0.6±0.1c	5±0bc	36.1±5.5c	0.46±0.1c
60	8.0±0.6a	3.8±0.4c	1.6±0.1b	1.5±0.2b	5±1b	50.8±6.6b	1.62±0.3b
75	8.6±0.8a	4.6±0.4b	1.8±0.2a	1.7±0.4b	8±2a	66.6±9.4a	1.80±0.2b
90	8.1±0.7a	5.4±0.7a	1.9±0.1a	2.5±0.1a	8±3a	69.2±7.4a	3.49±0.3a

Note: Different letters in the same column indicate significant differences at  $p=0.05$  level ( $n=8$ ) according to Duncan's test.

sowing. Although, LUE, EUE, and PY were all highest at 90 d after sowing, the difference between 75-90 d was not significant. EY was higher at 75 d after sowing than at 90 d. Both PY and EY were 1.75 times higher at 60 d after sowing than at 45 d. Both PY and EY

were only 1.2 times higher at 75 d after sowing than at 60 d. Seedlings meet the standards when the seedling period is 60 d, and from an economic perspective, the seedling period is recommended to be 60-75 d.

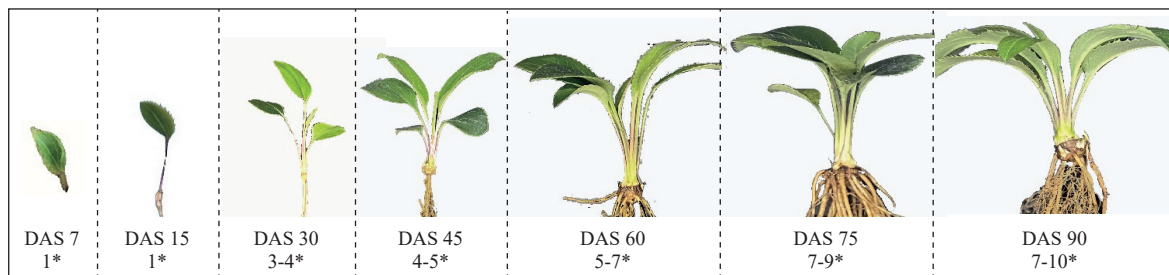
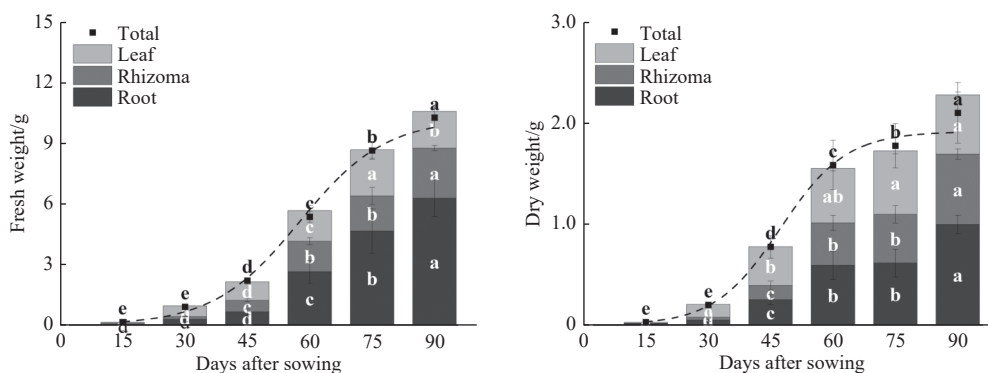


Figure 5 Seedling development of *A. Chinensis* in 32-cell deepened plug trays at 15, 30, 45, 60, 75, and 90 d after sowing



Note: Different letters in the same column indicate significant differences at  $p=0.05$  level ( $n=8$ ) according to Duncan's test

Figure 6 Fresh and dry weight of *A. Chinensis* seedlings measured at 15, 30, 45, 60, 75 and 90 d after sowing in 32-cell deepened plug trays

Table 5 Energy use efficiency of *A. Chinensis* seedlings measured at 15, 30, 45, 60, 75 and 90 d after sowing in 32-cell deepened plug trays

Day after sowing	LUE			EUE			PY/g·mol <sup>-1</sup>			EY/g·kWh <sup>-1</sup>						
15	0.004	±	0.001	d	0.001	±	0.000	d	0.204	±	0.043	e	1.155	±	0.242	e
30	0.012	±	0.001	c	0.005	±	0.000	c	0.606	±	0.165	d	3.435	±	0.935	d
45	0.032	±	0.004	b	0.012	±	0.002	b	0.963	±	0.096	c	5.460	±	0.542	c
60	0.035	±	0.004	b	0.013	±	0.002	b	1.688	±	0.123	b	9.572	±	0.697	b
75	0.043	±	0.005	a	0.016	±	0.002	a	2.017	±	0.224	a	11.436	±	1.268	a
90	0.042	±	0.006	a	0.016	±	0.002	a	1.949	±	0.090	a	11.049	±	0.511	a

Note: Different letters in the same column indicate significant differences at  $p=0.05$  level ( $n=8$ ) according to Duncan's test

#### 4 Conclusions

This study was conducted to determine the growth characteristics of *A. Chinensis* seedlings according to the cell size of plug trays and number of days after sowing. Plug tray cell size had a significant effect on the growth of *A. Chinensis* seedlings. Appropriate deepening of the plug tray significantly increased the stem diameter, leaf area, and rhizome fresh and dry weight of the seedlings, which were 1.4, 1.7, 1.7 and 1.4 times higher in the P32-D treatment than in the P32 treatment, respectively. In addition, energy use efficiency increased with increasing biomass accumulation of seedlings in deepened trays, with maximum PY and EY of 1.8 g/mol and 6.8 g/kWh for the P32-D treatment, respectively. Therefore, 32-cell deepened plugs are recommended for seedling production of *A. Chinensis*. In furthermore, in terms of the number of sowing days, the growth of shoots and rhizome was most vigorous at the 60 d after sowing, while the fresh weight of rhizome, which can be considered as an indicator of root growth,

increased steadily during the experiment but slowed down after 60 d. As a result, 32-cell deepened plug trays and a seedling nursery for a period of 60-75 d are recommended for commercial cultivation of *A. Chinensis* seedlings.

#### Acknowledgements

This research was supported by the earmarked fund for CARS (Grant No. CARS-21).

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