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EFFECTS OF SUBSTRATE RATIOS ON TRANSPLANTATION OF TISSUE-CULTURED GINGER SEEDLINGS

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Abstract

As the substrate for seedling growth, its physicochemical properties and nutrient composition directly affect the growth and development of plants after transplantation. However, there are few studies on the suitable substrate ratio for ginger tissue-cultured seedlings. This study aimed to systematically investigate the effect of different substrate ratios on the survival rate and growth of transplanted tissue-cultured seedlings of ginger (*Zingiber officinale* Rosc.). The garden soil, peat soil, perlite and vermiculite were used as substrates in the experiment. A total of nine groups of substrate ratio (J1-J9) were designed by using $L_9(3^4)$ orthogonal experimental design. The seedlings were comprehensively evaluated using physicochemical properties, nutrients content, growth status, defensive enzyme activities and reactive oxygen species (ROS) concentration, combined with the method of subordinate function for multi-dimensional evaluation. Results showed that a substrate consisting of garden soil, peat soil, perlite and vermiculite at a ratio of 2:2:3:1 was in the desirable range of bulk weight (0.441 g/cm³) and total porosity (71.47%). Its water-holding capacity (130.14%) was significantly higher ($P < 0.05$) than the other ratios and its nutrient balance (384.02 mg/kg of available phosphorus and 63.00% of organic matter) significantly contributed to the growth rate of plant height (367.78%), leaf length (12.27 cm) and SOD activity (168.20 U/g). Furthermore, the highest transplanting survival rate and affiliation function value were observed in this substrate ratio. The study revealed that the best survival rate and growth of ginger tissue-cultured seedlings were achieved under the ratio of garden soil: peat soil: perlite: vermiculite=2:2:3:1, and this result can provide technical support for the establishment of ginger tissue-cultured seedlings multiplication technology system and the application of low-cost production.

Key words: *Zingiber officinale* Rosc.; Tissue-cultured ginger seedlings; Substrate ratios; Survival rate; Growth and development

Introduction

Ginger (*Zingiber officinale* Rosc.), a perennial monocotyledonous rhizomatous plant of the Zingiberaceae family. It is primarily distributed in tropical and subtropical regions including Sri Lanka, Nepal, Bangladesh, Malaysia, India, and Southwest China (Tian *et al.*, 2020). China is the world's major ginger growing and exporting country, its ginger planting area exceeds 1/3 of the world's total planting area, and has the largest international trade volume accounting for 70% of the world's total trade (Dong *et al.*, 2023). Ginger is rich in nutrients, not only contains carbohydrates, proteins, a variety of vitamins and minerals, but also contains curcumin, ginger oil ketone, ginger enol and ginger alcohol, etc., can be used as a condiment or directly processed into ginger and other dry food, with detoxification, dispersal of cold, cough, vomiting and other pharmacological functions (Ismail *et al.*, 2016, Zhang *et al.*, 2021). Therefore, ginger, which is an important plant for both culinary and medicinal applications, has become a preferred crop for the revitalization of rural industries (Li *et al.*, 2018).

Traditional ginger cultivation is mainly based on asexual reproduction from rhizomes. However, ginger bacterial wilt represents the most widespread destructive pathogen of ginger and has been reported in several ginger growing countries (Prameela *et al.*, 2020). When infected seed ginger is used, crop yields are greatly reduced. For example, studies have shown that when Ralstonia solanacearum-infected rhizomes were used as seed ginger, yield losses were as high as 47% (Hu *et al.*, 2020). To eliminate pathogens from the source and reduce or even avoid the occurrence of ginger plague, more and more researchers have carried out research on the factory breeding technology of ginger tissue-cultured seedlings (Babu *et al.*, 2016, Zhao *et al.*, 2022a).

Seedling transplanting constitutes a key step in plant breeding production. The optimization of substrate ratios for different plants is a key factor for its success (Lei *et al.*, 2015). The synergistic effect of the physical structure and chemical properties of the cultivation substrate significantly influences plant root development and its nutrient uptake (Kuchkorova, 2025). Among the commonly used substrate materials, garden soil is low-cost but easy to slough, peat soil is rich in organic matter but needs to regulate pH, perlite can increase porosity but lacks nutrients, and vermiculite becomes a key component for water and fertilizer retention by virtue of its strong ion-exchange capacity (Fussy *et al.*, 2022, Schafer *et al.*, 2022). It has been shown that different cultivation substrates significantly affect the growth and survival of tomato (*Solanum lycopersicum* L.) and blueberry (*Vaccinium myrtillus* L.) seedlings (Ortiz-Delvasto *et al.*, 2023, Al-Khateeb *et al.*, 2024). Jae Kyung Kim found that apple rootstock seedlings had significantly high plant height, number of leaves, leaf area, stem fresh weight and root fresh

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weight under peat moss: vermiculite: perlite = 3:1:2 substrate ration (Kim *et al.*, 2021). Abdullah et al. reported that during the acclimatization of ginger (*Zingiber officinale var. rubrum*) tissue culture seedlings in a controlled environment, the survival rate was highest in a substrate mixture of topsoil, baked soil, and peat moss (2:1:1), while the mixture of peat moss and sand (1:1) resulted in the lowest survival rate (Abdullah *et al.*, 2023). Rezaei et al. found that using a 20 L pot and a substrate mixed from perlite and sand increased the yield of soilless ginger cultivation (Rezaei *et al.*, 2024). These results corroborate the existence of species-specific plant requirements for substrate rationing. Ginger, as a fleshy rhizome crop, requires both a loose and breathable substrate to prevent root rot and a continuous supply of mineral nutrients to promote seedling growth when transplanting tissue-cultured seedlings (Robinson *et al.*, 2025). However, most of the existing studies focus on the performance of a single substrate, overlooking the synergistic effects between of low-cost garden soil and functional materials.

Therefore, in this study, we used garden soil, peat soil, perlite, and vermiculite as substrates and mixed them in different proportions to form nine groups of substrates. By measuring the physical and chemical properties of the substrate, nutrient composition and plant growth, we explored the suitable substrate ratios for transplanting ginger tissue-cultured seedlings. The results of this study can provide technical support for the establishment of a technical system for the factory breeding of tissue-cultured seedlings and large-scale production of ginger.

Materials and Methods

Experimental materials: Tissue-cultured seedlings of "*Zingiber officinale* Rosc. Yujiang No.3", a widely cultivated ginger variety in Southwest China, were provided by the Research Institute of Specialty Plants of Chongqing University of Arts and Sciences. Cultivation substrates comprised garden soil (purple soil from Cucumber Hill, Yongchuan District), peat soil (Jilin Zhoujiabao Agricultural Development Co., LTD.), vermiculite (Shandong Lupan Horticultural Co., LTD.), perlite (Shandong Lupan Horticultural Co., LTD.), and peat soil (Jilin Zhoujiabao Agricultural Development Co., LTD.).

Experimental design: Orthogonal test method was used to design different ratios of substrates and observe the growth of ginger tissue-cultured seedlings under different substrate ratios (Li *et al.*, 2025). Garden soil, peat soil, perlite and vermiculite were selected as substrate factors, and nine groups (J1-J9) of substrate ratios were obtained according to the principle of L_9 (3^4) orthogonal experimental design by making orthogonal experimental table using SPSS software (version 27.0) (Table 1).

Transplantation of tissue culture ginger seedlings: The ginger tissue-cultured seedlings growing up to 5-6 leaves with lots of white roots and consistent health condition were selected for transplanting. The tissue-cultured seedlings were removed from the culture flasks with forceps, divided into single plants, and rinsed clean of any residual medium on the roots with water. Next, they were carefully transplanted into cavity trays with substrate, watered with sufficient rooting water, and shaded with shade netting after transplanting. Each group of substrate was cultivated with 15 plants and placed in a greenhouse (temperature between 22-25°C and relative humidity maintained at about 75%) for cultivation.

Table 1. Formula ratio of different cultivation substrates.

Group	Matrix			
	Garden soil	Peat soil	Perlite	Vermiculite
J1	1	1	1	1
J2	1	2	2	2
J3	1	3	3	3
J4	2	1	2	3
J5	2	2	3	1
J6	2	3	1	2
J7	3	1	3	2
J8	3	2	1	3
J9	3	3	2	1

These values represent the volume ratios of the factors in each matrix

Determination of physicochemical properties and nutrient content of cultivation substrates: Physical property measurements included the bulk weight, total porosity, and water holding capacity of the substrate, and chemical property measurements included the conductivity and pH of the substrate, which were determined following the method described by (Li *et al.*, 2023). The whole phosphorus, whole potassium and whole nitrogen contents of the cultivation substrate were determined by the sodium hydroxide alkali fusion-molybdenum antimony antimony colorimetric method, sodium hydroxide alkali fusion method, and Kjeldahl digestion method, respectively (Shu *et al.*, 2022). Fast-acting nitrogen was determined by alkali dissolved diffusion method, effective phosphorus by molybdenum-antimony colorimetry, fast-acting potassium by flame photometry, and organic matter by potassium dichromate oxidation-external heating method (Liu *et al.*, 2024b).

Growth index measurement: Plant height, leaf length, leaf width, and longest root length of tissue-cultured seedlings were determined by straightedge after transplanting, and stem thickness was determined by vernier caliper. The data were measured every 10 days. After sampling, the seedlings were divided into above-ground and below-ground parts, cleaned and placed in a constant temperature oven dried at 80 °C for 24 h until constant weight was achieved (drying was considered complete when the relative error between the last two mass measurements was ≤0.5%), and the biomass of above-ground and below-ground parts as well as the total biomass weight were determined.

Measurement of physiological and biochemical indicators: Superoxide dismutase (SOD) activity was measured using a Total Superoxide Dismutase Assay Kit from Shanghai Yuanye Biotechnology Co., Ltd. (China), following the NBT riboflavin colorimetric method. Catalase (CAT) activity was determined with a Catalase Assay Kit from the same manufacturer, using the UV colorimetric method. Malondialdehyde (MDA) content was quantified with a Plant Malondialdehyde Assay Kit (TBA method) from Shanghai Yuanye Biotechnology. Hydrogen peroxide (H_2O_2) content was assessed using a Hydrogen Peroxide Assay Kit from Sangong Bioengineering (Shanghai) Co., Ltd. (China), following the colorimetric method.. Assay methods and procedure followed the manufacturer's kit instructions.

Comprehensive evaluation of tissue culture seedling growth in different culture substrates: The indicators of ginger tissue-cultured seedlings under different cultivation substrates were subjected to affiliation analysis, and the following formulas were used for the calculation of the values of the affiliation function and the inverse affiliation function (Pathania *et al.*, 2024):

Value of the affiliation function: $U(X_j) = (X_j - X_{min})/(X_{max} - X_{min})$

Value of the inverse affiliation function: $U(X_j) = 1 - (X_j - X_{min})/(X_{max} - X_{min})$

X_j is the measurement index, X_{max} is the maximum value of the index in all treatments, and X_{min} is the minimum value. If the correlation is negative, the formula is calculated with the inverse membership function value. The value of each membership function was calculated, and the average value of the membership function was calculated and compared.

Data processing and analysis: The data were statistically analyzed, and affiliation function values were calculated. Graph plotting was performed using Origin 2021. All data were subjected to statistical analysis using SPSS (Version 27.0). One-way ANOVA was performed to determine significant differences among treatment means, followed by Duncan's multiple range test for post-hoc comparisons.

Results

Effect of substrate physicochemical properties on the growth of ginger tissue-cultured seedlings: There were significant differences in the physicochemical properties of the nine groups of substrates (Table 2). J3 exhibited the lowest bulk density (0.291 g/cm^3) and the lowest total porosity (69.11%). J5 demonstrated the highest water-holding capacity (130.14%), followed by J7 (128.55%) and J9 (92.66%). The electrical conductivity of J6 was the highest (2.98 mS/cm), followed by J9 (2.85 mS/cm), and J1 was the lowest (1.91 mS/cm). The pH value of J1 was the highest (7.68), and that of J9 was the lowest (6.99), all of which were close to neutral.

Comparison of nitrogen, phosphorus and potassium contents in different culture substrates: The data showed that J7 had the highest total nitrogen content of 1.309 g/kg, followed by J4, J9, and J8 (1.301 g/kg, 1212 g/kg, and 1.200 g/kg). J3 had the highest total phosphorus content of 17.19 g/kg, followed by J4 (17.00 g/kg). J9 had the highest total potassium content of 6.80 g/kg, followed by J8, J6 and J5 (6.63g/kg, 6.63g/kg, 6.51g/kg). J6 had the highest quick nitrogen content of 112.19mg/kg followed by J8 and J9 with 86.24mg/kg. J8 had the highest quick phosphorus content of 466.73mg/kg followed by J6 (401.74mg/kg). J9 had the highest quick J9 had the highest potassium content of 11.9mg/kg followed by J8 (11.3mg/kg). The organic matter content of J2, J5, J1 and J8 was above 60% with 63.90%, 63.00%, 60.38% and 60.31%, respectively (Table 3).

Effect of different substrate ratio on the growth of ginger tissue culture seedlings: The nine substrate ratios had different effects on the growth of ginger tissue-cultured seedlings (Table 4, Fig. 1). J6 exhibited the maximum root length of 21.73±1.21 cm, which was significantly different from the other groups of treatments ($p<0.05$). J5 had the longest stem thickness of 4.9±0.6 mm, which was significantly different from that of J3, J6, and J9 ($p<0.05$). J7 had the highest growth rate in plant height of 417.78±43.23%, followed by J5.J5 had the longest leaf length of 12.27±0.32 cm, which was 1.27 times longer than the longest leaf length of J7. J7 had the highest plant height growth rate of 417.78±43.23%, followed by J5. J5 had the longest leaf length of 12.27±0.32 cm, which was 1.27 times longer than the longest leaf length of J7. J3 had the largest leaf width of 1.72±0.03 cm, and the largest leaf widths of J2, J4, and J9 were significantly different from those of J3 ($p<0.05$). The aboveground and below-ground dry weight ratios of the tissue-cultured seedlings of J8 were the highest of 5.085±0.95, which was 20%-65% higher than the other groups. J2, J3, J5, J7, and J9 had the highest survival rate of 100%, followed by J8 (91.67%), and J6 had the lowest (75%).

Effect of different substrate ratio on antioxidant activity of ginger seedlings: The antioxidant property of plants can reflect the plant's ability to adapt to the external environment to a certain extent. SOD represents a key antioxidant enzyme in plants, which can eliminate free radicals generated during the metabolic process of plants, and its activity can reflect the physiological activity of plants as well as the resistance to adversity (Yuce *et al.*, 2024). As shown in Fig. 2A, J7 had the highest SOD activity (184.25 U/g), followed by that of J5 (168.20 U/g) and J9 had the lowest (74.16 U/g). CAT is also an important antioxidant enzyme in plants, which catalyzes the decomposition of hydrogen peroxide in plants and thus reduces the oxidative damage caused by hydrogen peroxide (Baba *et al.*, 2024). As shown in Fig. 2D J8 had the highest CAT content (331.67 U/g-min), followed by J2, J4, and J7 with 269.44 U/g·min, 254.33 U/g·min, and 241.11 U/g·min, respectively.

The level of H_2O_2 and MDA, as products of oxidative damage to the cell membrane, measures the degree of oxidation of the cell membrane and is a direct manifestation of plant stress (Shakya, 2024). The H_2O_2 content of J2 was the highest (3.345 μmol/g), and the H_2O_2 content was significantly different from that of other groups ($p<0.05$). The H_2O_2 content of J7 was the lowest, 0.618 μmol/g, with a significant difference (Fig. 2-C). The MDA content of J6 was the highest (1.853 μmol/mg), and the MDA content was significantly different from that of J6 in other groups ($p<0.05$). The MDA contents of J1 and J7 were lower than those of other treatments, which were 0.897 μmol/mg and 1.048 μmol/mg, respectively (Fig. 2-B).

Table 2. Physicochemical properties of different cultivation substrates.

Group	Bulk weight (g/cm^3)	Total porosity (%)	Water-holding capacity (%)	Electric conductivity (mS/cm)	pH value
J1	0.405 ± 0.050^{e}	75.02 ± 0.04^{d}	121.61 ± 4.57^{b}	1.91 ± 0.02^{g}	7.68 ± 0.09^{a}
J2	0.341 ± 0.019^{f}	78.54 ± 0.11^{c}	111.33 ± 0.61^{c}	2.28 ± 0.01^{e}	7.55 ± 0.16^{ab}
J3	0.291 ± 0.084^{g}	69.11 ± 1.67^{g}	96.58 ± 4.12^{d}	2.10 ± 0.04^{f}	7.38 ± 0.13^{bc}
J4	0.418 ± 0.094^{d}	72.01 ± 0.12^{ef}	97.78 ± 3.9^{d}	2.35 ± 0.04^{d}	7.34 ± 0.22^{bc}
J5	0.441 ± 0.073^{c}	71.47 ± 1.80^{f}	130.14 ± 7.36^{a}	2.28 ± 0.05^{e}	7.24 ± 0.13^{cd}
J6	0.416 ± 0.086^{de}	85.62 ± 0.35^{a}	112.87 ± 0.41^{c}	2.98 ± 0.02^{a}	7.02 ± 0.14^{d}
J7	0.496 ± 0.013^{b}	73.13 ± 0.63^{e}	128.55 ± 1.00^{a}	2.29 ± 0.02^{e}	7.12 ± 0.14^{cd}
J8	0.516 ± 0.025^{a}	79.85 ± 0.80^{bc}	108.83 ± 0.99^{c}	2.52 ± 0.03^{c}	7.05 ± 0.10^{d}
J9	0.430 ± 0.078^{c}	80.10 ± 0.27^{b}	92.66 ± 2.63^{d}	2.85 ± 0.05^{b}	6.99 ± 0.24^{d}

Different lowercase letters in the same column indicate significant differences between treatments ($p<0.05$)

Table 3. Nutritional components of different cultivation substrates.

Index	J1	J2	J3	J4	J5	J6	J7	J8	J9
Total nitrogen (g/kg)	0.999	0.830	0.942	1.212	1.192	1.134	1.309	1.200	1.301
Total phosphorus (g/kg)	15.49	15.35	17.19	17.00	15.00	14.05	14.72	13.10	12.16
Total potassium (g/kg)	6.29	5.36	5.48	5.64	6.51	6.63	6.11	6.63	6.80
Available nitrogen (mg/kg)	75.26	70.27	75.26	61.29	73.26	112.19	67.28	86.24	86.24
Available phosphorus (mg/kg)	336.76	301.31	283.58	313.12	384.02	401.74	384.02	466.73	389.93
Available potassium (mg/kg)	7.1	6.3	6.6	6.4	8.2	10.6	9.8	11.3	11.9
Organic matter (%)	60.38	63.90	57.62	59.90	63.00	54.37	57.02	60.31	50.14

Table 4. The growth status of ginger seedlings under different cultivation substrates.

Group	Maximum root length (cm)	Stem diameter (mm)	Growth rate of plant height (%)	Maximum leaf length (cm)	Maximum blade width (cm)	Dry weight ratio above and below ground	Survival rate (%)
J1	10.67 ± 0.57^{f}	4.3 ± 0.7^{abc}	327.53 ± 63.64^{bc}	10.50 ± 1.40^{bc}	1.67 ± 0.03^{ab}	4.075 ± 0.37^{b}	83.33
J2	12.83 ± 0.35^{cd}	4.2 ± 0.2^{abc}	302.64 ± 10.35^{bcd}	10.23 ± 0.32^{bc}	1.55 ± 0.1^{bc}	2.912 ± 0.45^{c}	100.00
J3	13.67 ± 0.67^{c}	3.8 ± 0.6^{bcd}	294.70 ± 24.11^{cde}	11.53 ± 0.42^{ab}	1.72 ± 0.03^{a}	2.560 ± 0.32^{cd}	100.00
J4	12.20 ± 0.57^{de}	4.3 ± 0.2^{ab}	242.27 ± 50.13^{de}	10.80 ± 0.10^{bc}	1.53 ± 0.25^{c}	2.503 ± 0.04^{cd}	83.33
J5	11.33 ± 0.95^{ef}	4.9 ± 0.6^{a}	367.78 ± 47.56^{ab}	12.27 ± 0.32^{a}	1.72 ± 0.08^{a}	2.162 ± 0.14^{de}	100.00
J6	21.73 ± 1.21^{a}	4.0 ± 0.4^{bcd}	229.16 ± 40.92^{e}	10.47 ± 1.00^{bc}	1.70 ± 0.05^{a}	1.772 ± 0.12^{e}	75.00
J7	9.95 ± 0.85^{f}	3.3 ± 0.3^{d}	417.78 ± 43.23^{a}	9.67 ± 0.45^{c}	1.73 ± 0.08^{a}	3.707 ± 0.60^{b}	100.00
J8	12.90 ± 1.48^{cd}	3.8 ± 0.1^{bcd}	241.62 ± 27.92^{de}	10.72 ± 0.89^{bc}	1.68 ± 0.08^{a}	5.085 ± 0.95^{a}	91.67
J9	16.70 ± 1.11^{b}	3.6 ± 0.2^{cd}	149.63 ± 43.68^{f}	9.90 ± 0.62^{c}	1.53 ± 0.1^{c}	2.246 ± 0.07^{cde}	100.00

Different lowercase letters in the same column indicate significant differences between treatments ($p<0.05$)

Table 5. Membership function values of different cultivation substrates on the growth indexes of ginger tissue culture seedlings.

Index	J1	J2	J3	J4	J5	J6	J7	J8	J9
Maximum root length	0.06	0.24	0.32	0.19	0.12	1.00	0.00	0.25	0.57
Stem diameter	0.61	0.54	0.28	0.63	1.00	0.43	0.00	0.28	0.15
Growth rate of plant height	0.66	0.57	0.54	0.35	0.81	0.30	1.00	0.34	0.00
Maximum leaf length	0.32	0.22	0.72	0.44	1.00	0.31	0.00	0.40	0.09
Maximum blade width	0.70	0.10	0.95	0.00	0.95	0.85	1.00	0.75	0.00
Dry weight ratio above and below ground	0.70	0.34	0.24	0.22	0.12	0.00	0.58	1.00	0.14
Survival rate	0.33	1.00	1.00	0.33	1.00	0.00	1.00	0.67	1.00
SOD enzyme	0.26	0.76	0.82	0.81	0.85	0.60	1.00	0.72	0.00
MDA enzyme	1.00	0.38	0.82	0.83	0.70	0.00	0.84	0.22	0.34
H_2O_2 content	0.31	0.00	0.88	0.64	0.47	0.37	1.00	0.60	0.20
CAT enzyme	0.11	0.80	0.03	0.75	0.16	0.20	0.70	1.00	0.00
Average membership function values	0.460	0.451	0.599	0.471	0.653	0.370	0.648	0.567	0.227
Ranking	**6**	**7**	**3**	**5**	**1**	**8**	**2**	**4**	**9**

Matrix analysis of correlation between different substrates and growth of ginger tissue-cultured seedlings and comprehensive evaluation of the affiliation function: The complex associations between substrate properties and plant physiological indicators were revealed by correlation matrix analysis (Fig. 3). Key correlations showed that pH was negatively correlated with most nutrients, suggesting that a higher pH under these experimental conditions may inhibit the availability and accumulation of certain nutrients. In contrast, total porosity and water-holding capacity were predominantly positively correlated with nutrient levels, indicating that a well-structured substrate is typically associated with higher fertility. Bulk density was negatively correlated with most growth indicators, underscoring the critical importance of a loose, porous physical structure for optimal plant growth. The dry weight ratio showed lighter-colored correlation squares with most other variables, indicating weaker correlations and suggesting that biomass allocation was relatively independent of the other measured substrate properties. A positive correlation was observed between SOD and CAT, indicating a synergistic increase in their activities in response to oxidative stress. The negative correlations between SOD/CAT and MDA content reflect the effectiveness of the antioxidant system in scavenging reactive oxygen species.

Fig. 1. Morphological growth of ginger tissue-cultured seedlings under nine different substrate ratios (J1–J9). Visual differences in plant height, leaf expansion, and overall vigor are evident among the treatments.

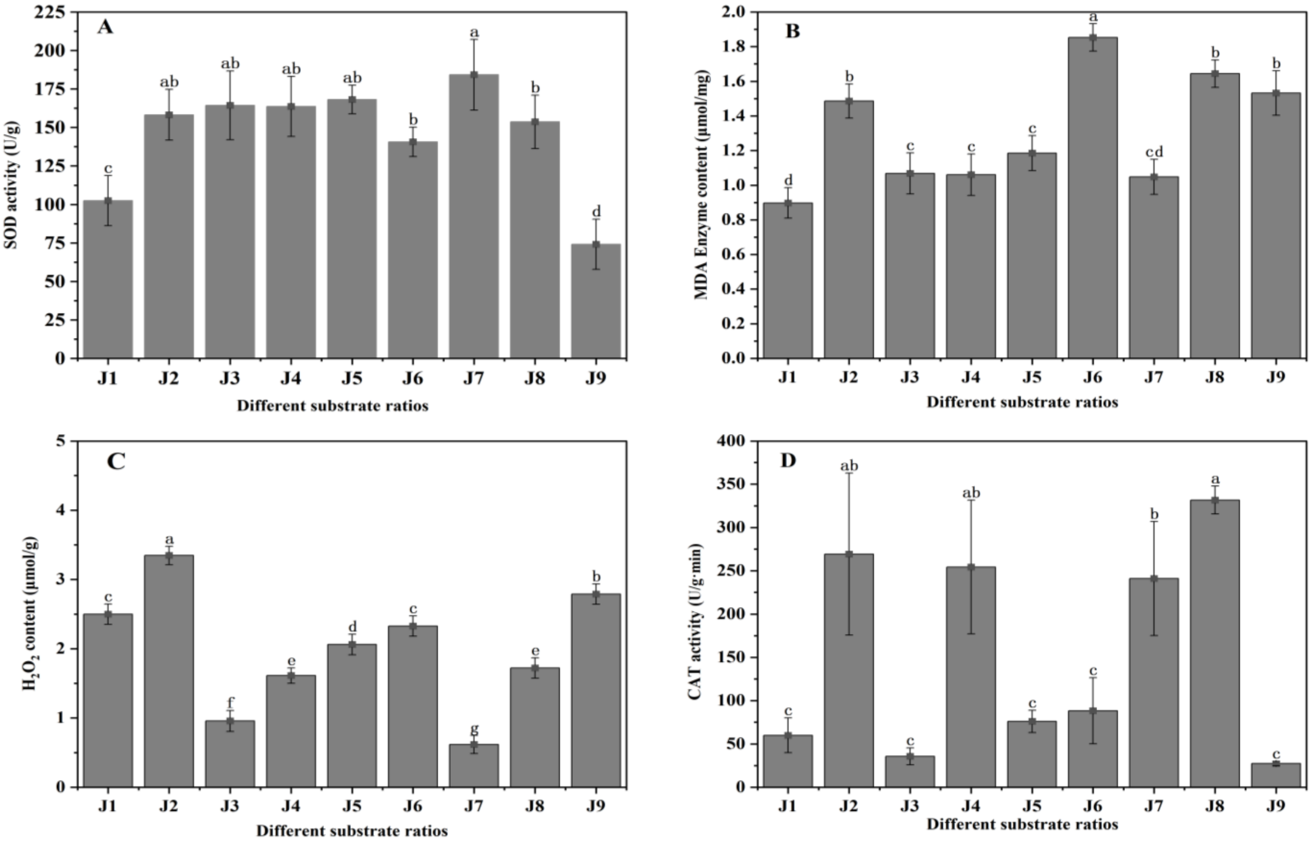

Fig. 2. Effects of different substrate ratios on antioxidant enzyme activities and oxidative stress markers in ginger tissue-cultured seedlings. (A) Superoxide dismutase (SOD) activity, (B) Malondialdehyde (MDA) content, (C) Hydrogen peroxide (H_2O_2) content, (D) Catalase (CAT) activity. Different lowercase letters above bars indicate significant differences among treatments ($p<0.05$).

The growth and physiological indexes of ginger tissue-cultured seedlings under different substrate ratios were comprehensively evaluated by using the fuzzy affiliation function evaluation method. J5 had the highest average affiliation function value of 0.653, followed by J7 with 0.648, which indicated that among the nine groups of substrate ratios, J5 was the most appropriate one for the cultivation of tissue-cultured seedlings out of the vials (Table).

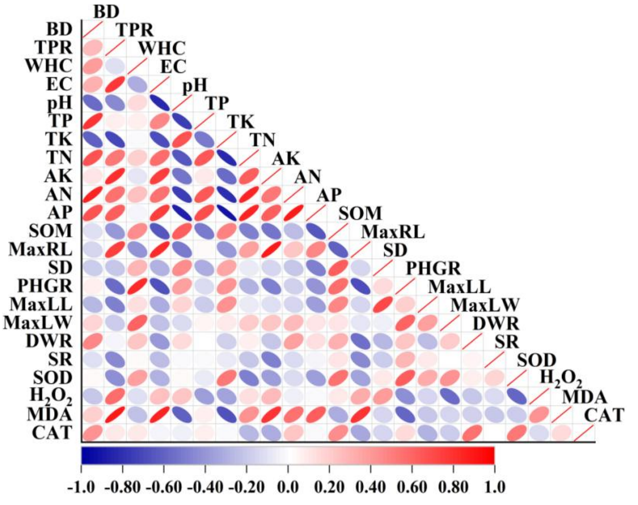

Fig. 3. Correlation heatmap analyzing the relationships between substrate properties, nutrient contents, and ginger seedling growth/physiological parameters. The color intensity and direction (blue for negative, red for positive) represent the strength and sign of the Pearson correlation coefficient between each pair of variables. (Abbreviations: BD: bulk density; TPR: total porosity; WHC: water-holding capacity; EC: electrical conductivity; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AN: available nitrogen; AP: available phosphorus; AK: available potassium; SOM: soil organic matter; MaxRL: maximum root length; SD: stem diameter; PHGR: plant height growth rate; MaxLL: maximum leaf length; MaxLW: maximum leaf width; DWR: dry weight ratio (above/below ground); SR: survival rate; SOD: superoxide dismutase; CAT: catalase).

Discussion

The successful transplantation of tissue-cultured seedlings is a critical bottleneck in commercial plant production, heavily dependent on the substrate's ability to provide physical support, water, nutrients, and a conducive root environment. This study systematically evaluated the impact of nine substrate ratios on ginger tissue-cultured seedlings, identifying J5 (2:2:3:1) as the optimal formulation through a comprehensive multi-index evaluation.

Effect of substrate physicochemical properties on the growth of ginger tissue-cultured seedlings: In terms of the physicochemical properties of the substrate, bulk density serves as an indicator of cultivation substrate density, which affects the growth of the plant root system and the retention of water and nutrients. Total porosity is a key factor affecting the permeability and water holding capacity of the cultivation substrate (Zhao *et al.*, 2022b, Liu *et al.*, 2024a). The bulk weight (0.441 g/cm³) and total porosity (71.47%) of J5 (garden soil: peat soil: perlite: vermiculite = 2:2:3:1) were in the appropriate range, and the water-holding capacity (130.14%) was significantly higher than the other ratios ($p<0.05$). Research indicates that root permeability increases when substrate bulk density is below 0.5 g/cm³, while total porosity in the range of 50%-95% helps to balance water and oxygen supply (Ma *et al.*, 2024). The water-holding capacity advantage of J5 may stem from the synergistic effect of the high water retention of peat soil and the pore structure of perlite, which provides a stable water environment for the root system and reduces water stress after transplanting. Meanwhile, its suitable electrical conductivity (2.28 mS/cm) with near-neutral pH (7.24) avoids high salt or extreme pH damage to the root system of ginger tissue-cultured seedlings. These findings align with those reported by Barbosa *et al.*, (Barbosa *et al.*, 2021). Further correlation matrix analysis showed Fig. 3 that total porosity, water holding capacity, electrical conductivity, and pH of the substrate were positively correlated with the growth indices of ginger tissue-cultured seedlings, such as root length, stem thickness, growth rate of plant height, and length of the longest leaf, which confirms the important influence of the physicochemical properties of the substrate on plant growth.

Association of substrate nutrient composition with growth of ginger tissue-cultured seedlings: Regarding the nutrient composition of the substrate, the elements of nitrogen, phosphorus and potassium are the main nutrients necessary for plant growth, and the organic matter can provide the nutrients needed for plant growth, and they affect the growth and development of plants (Yahaya *et al.*, 2023). In this study, J5 substrate had a high content of availble phosphorus (384.02 mg/kg) and organic matter (63.00%), which provided a rich nutrient base for it to promote the growth of ginger tissue-cultured seedlings. The efficient supply of availble phosphorus may have promoted the growth of leaves of ginger tissue-cultured seedlings (Table shows that the longest leaf length and leaf width of J5 were significantly higher than those of other groups). This was also confirmed by Liu *et al.*, in their study on the effect of phosphorus fertilizer on leaf traits in sapotaceae (Liu *et al.*, 2024b). It is worth noting that although J8 had the highest availble phosphorus content (466.73 mg/kg), its bulk weight (0.516 g/cm³) was significantly higher than that of the other groups resulting in decreased root aeration, limiting nutrient uptake efficiency, and ultimately the plant height growth rate (241.62%) was significantly lower than that of J5. In the correlation matrix analysis, bulk weight was negatively correlated with a number of growth indices such as the longest root length, stem thickness and plant height growth rate, further confirming the point. were negatively correlated, further confirming the point. This suggests that a single high nutrient content is not optimal and that synergistic optimization of physicochemical properties and nutrients is more critical. This is consistent with the findings of Negușie et al. (Negușier *et al.*, 2024). Conversely organic matter enhances substrate physical properties and increases the water and fertilizer holding capacity of the substrate (Saputra *et al.*, 2025). In this study, the water-holding capacity of J5 (130.14%) was significantly higher than that of the other groups, and the correlation matrix analysis revealed that organic matter was positively correlated with the water-holding capacity of the substrate. This indicates that the nutrients in the substrate not only directly affect plant growth, but also indirectly promote plant growth by improving the physical properties of the substrate (Hrebeckova *et al.*, 2024).

Comprehensive analysis of growth indexes of ginger tissue-cultured seedlings: The changes in plant growth may reflect not only the plant's ability to absorb water and nutrients, but also the plant's level of growth and health (Bin *et al.*, 2019). From the growth indexes of ginger tissue-cultured seedlings (Table), the plants under J5 substrate showed significant advantages. Their stem thickness, plant height growth rate (367.78%), maximum leaf length (12.27 cm) as well as maximum leaf width (1.72 cm) were significantly higher than the other groups. The growth rate of plant height and stem thickness reflected the growth rate of the plant after transplanting, while the leaf traits reflected the photosynthetic area and material production capacity of the plant (Sun *et al.*, 2024). Correlation analysis further revealed the intrinsic relationship between these growth indicators, for example, plant height growth rate was positively correlated with stem thickness, leaf length and leaf width, suggesting that the overall growth of the plant was harmonized.

Relationship between antioxidant properties and adaptation of ginger tissue-cultured seedlings: The nutrients of the substrate are involved in the regulation of the activity of many enzymes in the plant, and the enzyme activity of the plant is one of the strong evidences reflecting the functioning of the plant organism (Waqas *et al.*, 2018). SOD functions as a key antioxidant enzyme in plants, which can eliminate the free radicals produced by plant during metabolism, and its activity level can respond to the physiological activity and stress resistance of plant. CAT is also an important antioxidant enzyme in plants that catalyzes the decomposition of H_2O_2 in plants thereby reducing the oxidative damage caused by hydrogen peroxide. In addition, the levels of H_2O_2 and MDA content, as products of oxidative damage to cell membranes, provide a measure of the degree of oxidation of cell membranes (Shakeel *et al.*, 2019). The observed variations in antioxidant enzyme activities and oxidative stress markers among treatments highlight the physiological impact of substrate composition. Seedlings under the J5 formulation exhibited high SOD activity (168.20 U/g) coupled with moderate CAT activity, which contributed to effective ROS scavenging. This is reflected in the lower H_2O_2 and MDA contents in J5 compared to several other treatments, indicating reduced oxidative membrane damage. The superior physical structure of J5 (optimal aeration and moisture) likely mitigated root hypoxia and water stress, which are common inducers of ROS burst during transplantation. The high SOD activity in J7 (184.25 U/g) and its correlation with low H_2O_2 and MDA further substantiate the role of a robust antioxidant system in acclimatization. The negative correlation between SOD activity and MDA content (Fig. 3) underscores the importance of antioxidant capacity in maintaining cellular integrity. The molecular mechanisms underlying this substrate-induced priming may involve the upregulation of antioxidant gene expression mediated by phytohormonal signals from the roots, a hypothesis that warrants future investigation. The results indicated that the substrate ratios had an effect on the antioxidant properties of ginger tissue-cultured seedlings, and the reasonable substrate ratios could enhance the antioxidant capacity of the plants and improve their adaptability to the external environment. This is consistent with the results of Zhou *et al*. (Zhou *et al.*, 2025).

Beyond the physicochemical and nutritional factors assessed, the substrate microbiome likely plays a significant but unquantified role in seedling establishment. Different substrate compositions can select for distinct microbial communities, which in turn influence nutrient cycling, pathogen suppression, and plant hormone synthesis (Chen *et al.*, 2024). The inclusion of garden soil in our mixes introduced a diverse native microbiota, while peat and vermiculite may have contributed specific beneficial consortia. It is plausible that the optimal performance of the J5 ratio was partly facilitated by a more favorable rhizosphere microbiome that enhanced plant growth and stress resilience. The conclusion also aligns with the previous findings of Tuxun et al. (Tuxun *et al.*, 2025).

Conclusion

The study demonstrated that different substrate ratios significantly affected on the transplanting effect of ginger tissue-cultured seedlings. The substrate varies significantly in physicochemical properties and nutrient elements that affect the growth and development of ginger. The J5 ratio (garden soil: peat soil: Perlite: vermiculite=2:2:3:1) significantly increased the growth rate of plant height (367.78%), leaf length (12.27 cm), stem diameter (4.9 mm) and SOD (168.20 U/g) by optimising substrate physical structure (bulk density 0.441 g/cm^3, total porosity 71.47%, water-holding capacity 130.14%) and nutrient balance (available phosphorus 384.02 mg/kg, organic matter 63.00%). The correlation matrix analysis further confirmed the correlation between the physicochemical properties of the substrate, nutrients and physiological indices of plant growth. The J5 was identified as the optimal ratio, exhibiting the highest affiliation function value (0.653) through a comprehensive evaluation by the affiliation function method. This study confirmed that the J5 ration, based on the synergistic optimization of physicochemical-nutrient-physiological multi-dimensions could provide an efficient and cost-effective solution for the factory transplantation of ginger tissue-cultured seedlings.

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