Optimizing Photoautotrophic Micropropagation Conditions for Ginger

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Abstract

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a commonly available spice and medicinal plant. This study aimed to develop an alternative to conventional micropropagation using photomixotrophic micropropagation for ginger. Rootless ginger tissue culture plantlets were initiated in seedling trays grown in a chamber maintained under a 16 h/day photoperiod with $60 \mu\text{E m}^{-2} \text{s}^{-1}$ of cool white light from fluorescent lamps at $25 \pm 2^\circ\text{C}$ with an air relative humidity of $60 \pm 5\%$. The photoautotrophic micropropagation of ginger was optimized using vermiculite + peat (1:1(v/v)) as substrate combined with MS + 0.5 mg/L NAA + 0.1% Metalaxyl-M·Hymexazol + 80% relative humidity (RH). After 40 days of culture, in-vitro ginger seedlings were successfully bred with a rooting rate of 100.0%. The seedlings could be directly transplanted to the field without the need for domestication and cultivation of conventional tissue culture seedlings.

Introduction

Ginger (*Zingiber officinale* Rosc.) is an herbaceous perennial plant that has a long history of cultivation. It is used as a spice worldwide and has been an important ingredient in traditional Chinese medicine for over 2000 years (Zhou et al. 2020). In 2019, the global production of ginger was 4.08 million tons, which demonstrates its significant economic value in world trade (Li et al. 2021). Cultivated ginger is generally infertile and fails to set seed. Thus, it cannot be sexually propagated, so its rhizomes are used for vegetative propagation (Nair 2019). However, several types of soil-borne diseases can accompany rhizome 'seed' pieces. Bacterial wilt of ginger is the most widely destructive pathogen of ginger and has been reported in all ginger growing countries (Prameela et al. 2020). Crop yields can be greatly reduced when infested ginger rhizomes are used as seed. For instance, recent studies observed yield losses as high as 47% when *Pseudomonas solanacearum* infested sections of the rhizome were used as 'seed' in fumigated soil (Hu et al. 2020). Furthermore, the rhizome is the part of ginger that is sold to consumers. So, using a large proportion of ginger rhizomes as 'seed' for cultivation in the next growing season directly detracts from the supply to the market. Therefore, in-vitro propagation is a suitable alternative for the effective production of ginger that can also ensure that ginger seedlings do not carry pathogens.

The transition from tissue culture bottle seedlings to field seedlings is a major obstacle in ginger tissue culture technology. Transplantation survival directly determines whether tissue-cultured ginger seedlings can be utilized in field production. The survival rate of ginger tissue bottle seedlings transplanted in the field is low because they are delicate, their root system is weak, their regeneration ability is poor, and the microenvironment during the tissue culture stage is different than in the field. For ginger tissue culture seedlings to become field seedlings, they will need to go through four stages: in-bottle rooting, where the subculture cluster seedlings of ginger are divided into single plants and transferred to rooting medium; in-bottle refining, where the cap of the culture bottle of tissue culture seedlings with good roots is removed, a little water is added to the culture bottle, and it is moved out of the culture room after 3 days and cultured for about 10 days under natural light and temperatures of $20\text{--}28^\circ\text{C}$; in-greenhouse sending, where the refined tissue culture seedlings are planted in the seedbed of greenhouse at $20\text{--}28^\circ\text{C}$ with a relative humidity of 80% and shade from a shading mesh; and in-field rooting, where after 30–40 days of growth in the greenhouse seedbed and having established new roots, ginger seedlings are transplanted from the seedbed to the field (Zahid et al. 2021). The maximum percentage of in-vitro derived plants that survived acclimatization in the greenhouse was about 80% (Mohamed et al. 2011). To simplify this process, this study divided the successive cluster seedlings of ginger into single plants, and the field seedlings were directly established using photoautotrophic micropropagation technology. Photoautotrophic micropropagation helps the acclimatization phase, improving the anatomical and physiological characteristics that allow seedling survival and development (Santos et al. 2020). The

aim of this study was to develop a reliable and photoautotrophic micropropagation system that would improve the quality of ginger tissue culture seedlings and increase the survival rate during transplantation.

Materials And Methods

Plant material preparation

‘Laifeng’ ginger (*Zingiber Ofcinale* Roscoe) is a high-quality ginger cultivar planted in Laifeng County, Enshi, Huhei, China, and is a national product of Geographical Indication originated. ‘Laifeng’ ginger plantlets were previously established in vitro from meristem cultures and maintained in Murashige Skoog (MS) medium (Murashige and Skoog 1962) with 1.0 μM 6-benzylaminopurine (6-BA) and 0.5 μM α-naphthaleneacetic acid (NAA) for cluster bud multiplication. For micropropagation, the obtained plantlets were subculture in the above medium. These propagation procedures were carried out so that the necessary number of single shoots with similar morphologies could be obtained. Cultures were maintained at 25 ± 2°C with a 16 h per day photoperiod at a light intensity of 60 μE m⁻² s⁻¹ (Zhou et al. 2020). The breeding vial seedlings from the 30-day ‘Laifeng’ ginger proliferation culture were used as experimental material for further study and divided into single plants (plant height 3–5 cm, stem thickness ≥ 0.2 cm).

Treatments and in vitro culture conditions

The rootless ginger tissue culture seedlings were placed in seedling trays (size 35–50 cm × 15–20 cm × 10–15 cm) with transparent covers and trays. The seedling trays contained 8–10 cm thick culture substrates. The corresponding solution and antimicrobial agents were mixed into the culture substrates according to a specific humidity (Table 1). After planting, the seedling trays were covered so that the seedlings were in relatively closed environments (Fig. 1a). The seedlings grew roots after 20 d and the covers of the seedling trays were removed (Fig. 1b). When the substrate was dry, 1–2 cm of tap water was added to the underlying trays. The plants were cultivated for 20 d before they were directly planted in the field (Fig. 1c). The seedling trays were placed in a growth chamber and maintained under 16 h/day light conditions at an intensity of 60 μE m⁻² s⁻¹ from cool white fluorescent lamps at 25 ± 2°C with an in air relative humidity (RH) of 60 ± 5%.

Table 1
Different factors were designed to study the effects on the growth of rootless ginger tissue culture seedlings

Treat	Substrate	Nutrition liquid	RH levels	Antimicrobial agents
T1	?	MS+0.5mg/L NAA	80%	0.1% Metalaxyl-M·Hymexazol
T2	vermiculite:peat (1:1(v/v))	?	80%	0.1% Metalaxyl-M·Hymexazol
T3	vermiculite:peat (1:1(v/v))	MS+0.5mg/L NAA	?	0.1% Metalaxyl-M·Hymexazol
T4	vermiculite:peat (1:1(v/v))	MS+0.5mg/L NAA	80%	?

Data collection and statistical analysis

The plant height was measured vertically from the shoot–root junction to the highest new leaf. Stem thickness was measured by vernier caliper. The fresh weights of leaves, stems, and roots of ginger plants were measured. The dry weights of the ginger plants were measured after drying in a drying oven at 80°C for 3 d. These botanical characteristics of the rootless ginger plantlets were studied under various conditions to examine the effects of

different substrates (Table 2), different nutritional liquids (Table 3), different humidities (Table 4), and different antibacterial agents (Table 5). Each treatment was replicated three times with 10 explants in each replicate.

Table 2
Effect of different substrates on ginger seedlings growth

Substrates	Plant height (cm)	Stem diameter (mm)	Number of roots (Explant)	Leaf weight (g)	Stem weight (g)	Root weight (g)	Fresh weight (g)	Dry weight (g)
general vegetable seedling-raising substrates	7.730±	3.801±	4.933±	0.905±	1.359±	0.450±	2.617±	0.204±
	1.255 b	0.448 a	1.569 c	0.135 cd	0.072 b	0.030 e	0.183 d	0.016 c
Peat	9.093±	3.997±	6.733±	1.199±	1.850±	1.491±	4.361±	0.321±
	1.569 a	0.404 a	2.594 b	0.039 b	0.212 a	0.341 a	0.322 a	0.027 a
coconut bran	8.313±	3.813±	6.867±	1.019±	2.019±	0.978±	3.963±	0.248±
	0.814 ab	0.378 a	2.029 b	0.089 c	0.260 a	0.153 c	0.111 b	0.008 b
vermiculite	6.980±	3.248±	9.000±	0.807±	1.106±	0.680±	2.576±	0.193±
	0.989 c	0.465 b	2.875 a	0.115 d	0.124 c	0.071 d	0.274 d	0.019 c
vermiculite:peat (1:1(v/v))	9.940±	3.921±	5.000±	1.377±	1.926±	1.460±	4.100±	0.312±
	2.027 a	0.372 a	1.966 c	0.113 a	0.349 a	0.184 a	0.875 ab	0.018 a
Perlite:vermiculite (1:1(v/v))	7.540±	3.376±	8.533±	0.875±	1.300±	1.147±	3.238±	0.233±
	1.113 b	0.447 b	3.096 a	0.068 d	0.118 b	0.070 b	0.110 c	0.003 b
Values are means±standard error (n=10). Means followed by the same letters in each column are not significantly different at p < 0.05 using Duncan's multiple range test (DMRT). The same below.								

Table 3
Effects of different nutrition liquid on ginger seedlings growth

nutrition liquid	Plant height (cm)	Stem diameter (mm)	Number of roots (Explant)	Leaf weight (g)	Stem weight (g)	Root weight (g)	Fresh weight (g)	Dry weight (g)
H ₂ O	6.417±	3.399±	4.083±	0.834±	0.871±	0.381±	2.082±	0.163±
	1.126 b	0.518 a	0.862 b	0.027 c	0.204 b	0.021 c	0.246 c	0.017 cd
0.5MS	8.217±	3.324±	4.000±	1.122±	1.037±	0.319±	2.452±	0.179±
	1.364 a	0.544 a	1.080 b	0.055 a	0.175 b	0.106 d	0.342 b	0.018 bc
0.5MS+0.5 mg/L NAA	7.508±	2.987±	2.667±	0.952±	0.900±	0.188±	2.038±	0.150±
	1.237 ab	0.444 b	1.106 c	0.137 b	0.185 b	0.052 e	0.256 c	0.017 d
0.5MS+1.0 mg/L NAA	6.793±	3.021±	8.400±	0.760±	0.988±	0.759±	2.421±	0.191±
	1.346 b	0.506 b	3.241 a	0.051 d	0.143 b	0.071 a	0.156 b	0.002 b
MS+0.5mg/L NAA	8.193±	3.047±	6.800±	1.152±	1.912±	0.531±	3.506±	0.290±
	0.692 a	0.436 b	3.591 ab	0.146 a	0.194 a	0.065 b	0.333 a	0.065 a
MS+1.0 mg/L NAA	6.813±	2.871±	8.733±	0.668±	0.970±	0.408±	2.045±	0.156±
	1.199 b	0.450 b	3.193 a	0.050 e	0.194 b	0.102 c	0.210 c	0.013 d

Table 4
Effects of substrates with different RH on ginger seedlings growth

Treat	Plant height (cm)	Stem diameter (mm)	Number of roots (Explant)	Leaf weight (g)	Stem weight (g)	Root weight (g)	Fresh weight (g)	Dry weight (g)
60%	7.517±	2.913±	2.667±	0.764±	0.773±	0.164±	1.699±	0.130±
	1.035 b	0.465 b	1.312 a	0.074 a	0.019 a	0.029 b	0.034 ab	0.005 a
70%	7.483±	3.305±	2.333±	0.689±	0.774±	0.156±	1.545±	0.123±
	1.053 b	0.248 a	0.745 a	0.052 b	0.228 a	0.062 b	0.220 b	0.011 a
80%	8.133±	3.060±	2.583±	0.788±	0.784±	0.208±	1.775±	0.123±
	0.751 a	0.341 b	0.954 a	0.069 a	0.066 a	0.043 a	0.083 a	0.012 a
90%	7.475±	3.178±	2.500±	0.765±	0.822±	0.170±	1.750±	0.123±
	0.947 b	0.393 ab	0.957 a	0.035 a	0.081 a	0.024 b	0.131 a	0.013 a

Table 5
Effects of different antimicrobial agents on ginger seedlings growth

Treat	Plant height (cm)	Stem diameter (mm)	Number of roots (Explant)	Leaf weight (g)	Stem weight (g)	Root weight (g)	Fresh weight (g)	Dry weight (g)
0.1% sodium hypochlorite	7.700±	3.003±	2.750±	0.692±	0.965±	0.046±	1.683±	0.118±
	1.197 a	0.653 b	1.299 b	0.036 a	0.170 a	0.019 d	0.232 a	0.016 a
0.2% sodium hypochlorite	7.433±	3.415±	3.000±	0.613±	0.780±	0.086±	1.479±	0.126±
	0.974 a	0.499 a	1.528 b	0.068 b	0.055 b	0.033 b	0.140 ab	0.018 a
0.3% sodium hypochlorite	6.433±	2.961±	0.917±	0.569±	0.796±	0.015±	1.374±	0.102±
	0.788 b	0.531 b	0.954 d	0.081c	0.150 b	0.007 e	0.234 b	0.020 b
0.3% Isothiazolinone	6.583±	2.515±	0.667±	0.482±	0.625±	0.003±	1.101±	0.104±
	0.975 b	0.452 c	1.027 d	0.043 d	0.026 c	0.004 f	0.061 c	0.006 b
0.2% Carbendazim	7.550±	3.044±	2.333±	0.624±	0.816±	0.099±	1.556±	0.106±
	0.900 a	0.665 b	0.943 c	0.051 b	0.086 b	0.026 b	0.033 a	0.006 b
0.1% Azoxystrobin	6.750±	2.645±	4.000±	0.585±	0.710±	0.146±	1.438±	0.117±
	1.019 b	0.416 c	1.472 a	0.053 bc	0.073 b	0.024 a	0.113 b	0.012 a
0.3% Resin acid copper salt	7.217±	2.635±	2.667±	0.602±	0.702±	0.067±	1.368±	0.092±
	1.168 a	0.380 c	1.179 b	0.008 b	0.080 b	0.028 c	0.074 b	0.008 b
0.2% Chlorobromoisocyanurate	7.083±	2.752±	1.833±	0.561±	0.777±	0.049±	1.384±	0.103±
	0.931 ab	0.526 bc	0.799 c	0.036 c	0.083 b	0.013 d	0.107 b	0.010 b
0.1% Metalaxyl-M-Hymexazol	6.900±	2.924±	4.000±	0.597±	0.926±	0.132±	1.654±	0.132±
	0.913 ab	0.361 b	1.958 a	0.053 bc	0.061 a	0.040 a	0.136 a	0.014 a

A completely randomized design was applied for all experiments. The data were assessed using analysis of variance (ANOVA) in the statistical analysis software (SAS) version 9.4 and differences between means were identified using Duncan's multiple range test (DMRT) at $p < 0.05$.

Results

Effect of different substrates on ginger seedlings growth

Ginger seedlings were cultured in different substrates (Table 2) to study the effect of substrate on growth. In general, the peat treatment was conducive to the growth of ginger rootless tissue culture seedlings, while the vermiculite treatment was conducive to rooting (Table 2). The highest number of plants per shoot (9.940 ± 2.027 cm) was observed in the treatment with vermiculite + peat. The lowest number of plants per shoot (6.980 ± 0.989 cm) occurred in the vermiculite only treatment. Table 2 shows that the plant height was 9.940 ± 2.027 cm, stem diameter was nearly 4 mm, number of roots was 5.000 ± 1.966 per explant, leaf weight was 1.377 ± 0.113 g, stem weight was 1.926 ± 0.349 g, root weight was 1.460 ± 0.184 g, total fresh weight was 4.100 ± 0.875 g, and dry weight was 0.312 ± 0.018 g when the substrate had a vermiculite to peat ratio of 1:1 (v/v).

Effects of different nutritional liquids on ginger seedling growth

Water, Ms solution, and MS solution + hormone were added to the substrates to study their effects on the number of roots and other botanical characteristics of rootless ginger tissue culture seedlings. Among the substrates with different nutritional liquids, there was a significant increase in stem weight, fresh weight, and dry weight in plantlets grown on substrate with MS + 0.5 mg/L NAA (Table 3). The absence of hormone in the substrate resulted in larger stem diameters and the addition of 1.0 mg/L NAA resulted in increased rooting. In general, growth when water was used as the nutritional liquid was slower than when MS solution was used. Interestingly, in the process of inducing plant rooting, the hormone concentration was not directly proportional to rooting efficiency. Therefore, different concentrations of hormone were added to the substrate, and the results showed that when 0.5 mg/L NAA was added, plant height and leaf weight were significantly higher than when 1.0 mg/L NAA was added.

Effects of substrates with different RH on ginger seedlings growth

All ginger plantlets grew normally and vigorously under the different RH and the survival rate of the ginger seedlings was 100% in all treatments. There were no significant differences among RH treatments in number of roots, stem weight, or dry weight (Table 4). However, root weight was significantly greater in the RH 80% substrate than the other RH treatments. When the RH was 60%, the plant height (7.517 ± 1.035 mm), stem diameter (2.913 ± 0.465 mm), and root weight (0.164 ± 0.029 g) were lower than RH 80%, but the number of roots (2.667 ± 1.312 per explant) was higher. When the substrate RH reached 90%, the plant height (7.475 ± 0.947 mm), stem diameter (3.178 ± 0.393 mm), and root weight (0.170 ± 0.024 g) were relatively low. Based on the above analysis, the optimum substrate RH was 80%.

Effects of different antimicrobial agents on ginger seedlings growth

Rootless ginger tissue culture seedlings were planted in non-sterilized substrates and held in a closed culture environment with a high temperature and high humidity, which are conditions that facilitate the growth of microorganisms. In order to avoid harm to ginger seedlings from microorganisms, we added antimicrobial agents into the substrate. These antimicrobial agents were capable of inhibiting 100% of microorganism growth in rootless tissue culture seedlings and field seedlings. The data in Table 5 show significant differences ($p < 0.05$) among the applied different treatments. These data also revealed negative relationships between increasing sodium hypochlorite concentration and plant height, root number, fresh weight, and botanical characteristics (Table 5). Therefore, the bacteriostatic agent inhibited the growth of ginger seedlings in addition to inhibiting microorganisms. The maximum number of roots (4.000 ± 1.472 per explant) was recorded when 0.1% Metalaxyl-M·Hymexazol was used, followed by when 0.1% Azoxystrobin was used, but the two were not significantly different. In this study, the most suitable antimicrobial agent was 0.1% Metalaxyl-M·Hymexazol.

Discussion

The rhizome of ginger is used for vegetative propagation because it is an unfertile species (Nair 2019). The rhizome is also the part of ginger used as a commercial product, so the ginger rhizomes used as 'seed' for cultivating in the next growing season will detract from its supply in the market. Besides, soil-borne pathogens such as bacterial wilt (*Pseudomonas solanacearum*), rhizome rot disease (*Pythium myriotylum*, *Pythium spinosum*, and *Pythium sylvaticum*), and nematodes (*Meloidogyne spp.*) are easily transmitted during vegetative reproduction, carried by the ginger rhizomes (Kasilingam et al. 2018; Abed et al. 2020). We all know that in-vitro propagation not only could be a suitable alternative for the effective production of ginger seedlings, but also can eliminate the transmission of pathogens through the ginger rhizomes. Therefore, ginger tissue culture seedlings not only solve the problem of using a large proportion of marketable ginger as seed, but also ensures disease-free of ginger seeds. At the same time, the asexual propagation ginger for producing germplasm for breeding purposes has proven difficult, but we can use tissue culture technology to mutate ginger in vitro, so as to produce new germplasm (Jagadev et al. 2008; Mohanty et al. 2008; Zhou et al. 2020). However, the cost of transplanting cultured seedlings to the field accounts for 40–60% of the total costs of the tissue culture seedling production process. Therefore, improving the survival rates of ginger tissue culture seedlings during the transplantation stage and reducing production costs are urgently needed so that tissue culture technology can be applied to commercial production.

For transplantation, the in-vitro grown ginger plantlets were thoroughly washed with tap water to remove residual agar from roots. Then they were immersed in 0.2% aqueous Bavistin solution (fungicide) for 15–20 min and washed with tap water. The treated plantlets were then transplanted to pots filled with the different substrates and cultured in a greenhouse (Mohamed et al. 2011). Mohamed et al. (2011) found that the acclimatized rooting ginger tissue culture seedlings could be domesticated in peatmoss + sand + vermiculite with a survival rate of 60%. The regenerated ginger plantlets were then planted in a potting mixture of equal proportions garden soil, sand, and vermiculite and had an 85% survival rate (Samsudeen et al. 2000). Hung et al. (2018) found that when *Dendrobium officinale* could be acclimatized in plastic pots containing a mixture of 1:1 humus soil and coconut bran, the survival rate was about 31%. However, in this study, the rootless ginger tissue culture seedlings in seedling tray were incubated in a growth chamber and successfully acclimatized with 100% survival.

Among the six substrates selected in this study, the vermiculite + peat (1:1(v/v)) substrate was the most suitable. This may have been because vermiculite facilitates good air permeability and water absorption, while peat is rich in organic matter and humic acids. Although there were many substrates suitable for the cultivation of ginger tissue culture seedlings, such as peat + vermiculite + sediment (1:1:1(v/v/v)) (Qiu et al. 2020), in-vitro rooted 'Bentong' ginger plantlets were acclimatized in a growing media mixed of soil + coco peat + vermiculite (1:1:1(v/v/v)) (Zahid et al. 2021). The compositions of the soil and sediment were not determined, but the peat and vermiculite were known. Therefore, the substrate formulation of this study is easier to commercialize.

This obtained result was in line with the findings of Jagadev et al. (2008), who observed that for rooting of *Z. officinale* Rosc, MS supplemented with NAA (0.5 mg/L) was more effective and resulted in the maximum number of roots per shoot. Different nutritional liquids also affected the rooting rate. There have been many reports in this regard, such as Rout et al. (2008) who indicated that excised shoots were rooted on half-strength MS basal salts supplemented with 0.25 mg/l IBA or IAA and 20 g/l (w/v) in *Acacia chundra*. Kambaska et al. (2009) concluded that in-vitro shoots of *Z. officinale* Rosc rooted best when half strength MS basal medium supplemented with 2.0 mg/L NAA was used. Mohamed et al. (2011) found that shootlets became highly rooted when half strength B5 medium was supplemented with 1.0 mg/L NAA. In-vitro root induction in ginger is further enhanced by supplementing the culture medium with different nutrients (Abbas et al. 2011; Mehaboob et al. 2019). Determining the optimum type and concentration of nutrition can significantly enhance the in-vitro root induction of ginger and facilitate the

acclimatization and successful establishment of the in-vitro plantlets in field conditions (Mehaboob et al. 2019; Jualang et al. 2015). In this study, the plant height and root number were optimized when rootless ginger tissue culture seedlings were planted with MS + 0.5 mg/L NAA.

Loose and breathable substrate combined with appropriate humidity is necessary for rooting in the process of photoautotrophic micropropagation. Too much water and low oxygen content in the substrate are not conducive to the respiration of the plant and can easily cause base rot and reduce the rooting rate. However, low water contents in the substrate can make it difficult for plants to obtain enough water from the substrate, resulting in plant water loss, which can affect metabolism and result in a low rooting rate. Proper humidity can keep the plant moist, provide water for metabolism, and ensure the supply of oxygen to a certain extent. This is an important measure for maximizing the rooting rate. The goal of this study was to seek the optimum relative humidity (RH) levels for ginger photoautotrophic micropropagation. The results indicated that a moderate RH level of 80% was best because it had the best rooting effect for rootless tissue culture seedlings. The plant height and stem diameter of rootless ginger tissue culture seedlings were lowest in the 60% RH treatment, and the plant height, stem diameter, and root weight were relatively low in the 90% RH treatment. Previous studies have reported that a moderate level of water stress may actually be necessary to initiate root formation and optimize rooting success (Lebude et al. 2004, Tombesi et al. 2015). This study supports the previous reports that intermediate RH levels may actually promote root formation (Tyler et al. 2017)

Microbial contamination of plants can adversely affect plant tissue culture. These microbes can compete for nutrients, increase culture mortality, and may result in variable growth, tissue necrosis, and reduced shoot proliferation and rooting (Afolabi, 2009). In this study, rootless tissue culture seedlings of ginger were planted in vermiculite and peat supporting material, which were sugar free and had almost no bacterial or fungal contamination. However, because of the semi-enclosed and high humidity environment, some microbial growth did occur. Adding 0.1% Metalaxyl-M·Hymexazol to the substrate during the photoautotrophic micropropagation of ginger effectively inhibited the growth of bacteria. Furthermore, a certain concentration of Metalaxyl-M·Hymexazol not only had an antibacterial effect, but also promoted the rooting of ginger seedlings.

Micropropagation is an advanced technology capable of producing a large number of genetically superior and pathogen-free plants rapidly and in a small amount of space. However, the widespread application of micropropagation is still limited by high production costs, which are mostly attributed to the significant loss of plants grown in vitro due to microbial contamination, poor rooting, and low survival rates during the ex-vitro acclimatization stage (Kozai et al. 2001). Labor costs for rooting and acclimatization of plantlets account for approximately 60% of the total production costs in conventional micropropagation. Furthermore, there is relatively high mortality in plantlets due to the extreme environmental stresses experienced during the acclimatization stage. In-vitro grown explants and plantlets have been considered to have relatively low photosynthetic ability and to require sugar as a carbon and energy source for their heterotrophic or mixotrophic growth. Therefore, it is important that studies focus on optimizing the nutrient medium in vitro and mixotrophic micropropagation for specific plants and on determining when and how the medium should be applied to explants/plantlets in vitro. In this study, photoautotrophic micropropagation (PAM) of ginger was optimized using vermiculite + peat (1:1(v/v)) as substrate combined with MS + 0.5 mg/L NAA + 0.1% Metalaxyl-M·Hymexazol + 80% RH. After 40 days of culture, ginger seedlings grown in vitro were successfully bred with a rooting rate of 100.0%.

Declarations

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Author contributions

WJP planned and designed the research. GFL, ZJ, FJP and XY performed the experiments. WJP, QCD and ZJ analyzed the data. WJP and ZJ wrote the manuscript.

Compliance with ethical standards

Conflict of interest

The authors confirm that this article content has no conflict of interest.

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Figures

Figure 1

Rooting in vitro of rootless tissue culture seedlings **a** The rootless tissue culture seedlings of ginger were cultured in culture vessels containing 8.0-10.0 cm thick mixed substrates and the top of the culture vessels was covered with transparent materials. **b** The cover on the culture vessels was removed after 15-20 d. **c** Ginger seedlings can be transplanted in the field.