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 conventional micropropagation using photomixotrophic micropropagation for ginger. Rootless ginger tissue culture plantlets were initiated in culture vessels grown a chamber maintained under a 16 h/day photoperiod with an intensity of $60 \mu\text{E m}^{-2} \text{s}^{-1}$ from cool white fluorescent lamps at $25 \pm 2^\circ\text{C}$ with an air relative humidity of $60 \pm 5\%$. 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%.

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 and 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 the 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. Surviving transplantation will directly determine whether tissue-cultured ginger seedlings can be utilized in field production. The survival rate of ginger tissue bottle seedlings transplanted in the field will be 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 (The subculture cluster seedlings of ginger were divided into single plants and transferred to rooting medium.), in-bottle refining (Remove the cap of the culture bottle of tissue culture seedlings with good roots, put a little water in the culture bottle, move out of the culture room after 3 days, and culture for about 10 days under natural light and temperature of $20\text{--}28^\circ\text{C}$.), in-greenhouse sending (The tissue culture seedlings after refining were planted on the seedbed of protected cultivation. The temperature in the shed is $20\text{--}28^\circ\text{C}$, the relative humidity is 80%, and the shed top is covered with shading net.), and in-field rooting (After 30–40 days of planting on the greenhouse seedbed, new roots will grow, and ginger seedlings will be transplanted from the seedbed in the field.) (Zahid et al. 2021). The maximum percentage of in-vitro derived plants that have survived acclimatization in a greenhouse was about 80% (Mohamed et al. 2011). In view of the above problems, the successive cluster seedlings of ginger were divided into single plants, and the field seedlings were directly formed by photoautotrophic micropropagation technology in this study. 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 transplanting.

Materials And Methods

Plant material preparation

"Laifeng" ginger plantlets were previously established in vitro from meristem cultures and cultivated in Murashige Skoog (MS) medium (Murashige and Skoog. 1962) with $1.0 \mu\text{M}$ 6-benzylaminopurine (6-BA) and $0.5 \mu\text{M}$ α -naphthaleneacetic acid (NAA) for cluster bud multiplication. For micropropagation, the obtained plantlets were then transferred to 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 $25 \pm 2^\circ\text{C}$ with a 16 h per day photoperiod at a light intensity of $60 \mu\text{E m}^{-2} \text{s}^{-1}$ (Zhou et al. 2020). The breeding vial seedlings from the 30-day "Laifeng" ginger proliferation culture were used as test material and divided into single plants (plant height 3–5 cm, stem thickness ≥ 0.2 cm).

In-vitro culture conditions

The rootless ginger tissue culture seedlings were placed in culture vessels (size $35\text{--}50 \text{ cm} \times 15\text{--}20 \text{ cm} \times 10\text{--}15 \text{ cm}$) containing mixed substrates $8.0\text{--}10.0 \text{ cm}$ thick. The culture vessels had water absorption holes at the bottom and trays below. After planting, the tops of the culture vessels were covered with a transparent material so that the seedlings were in relatively closed environments (Fig 1a). The seedlings grew roots after culturing for 20 d, at which point the cover on the culture vessels was removed (Fig 1b). When the substrate was dry, 1–2 cm of water was added to the underlying trays. The plants were cultivated this way for 20 d before they were directly planted in the field (Fig 1c). All culture vessels were placed in a growth chamber and maintained under 16 h/day light conditions at an intensity of $60 \mu\text{E m}^{-2} \text{s}^{-1}$ from cool white fluorescent lamps at $25 \pm 2^\circ\text{C}$ with an in air relative humidity (RH) of $60 \pm 5\%$.

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, different solutions, different antibacterial agents, and different soil humidities. Each treatment was replicated three times, with 10 explants in each replicate.

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 media of different formulations to examine how different substrates influenced 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 1). 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 1 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 solutions on ginger seedlings growth

Determining the optimum type and concentration of auxin can significantly enhance the induction of in-vitro root growth in ginger, which will subsequently help with the establishment of in-vitro-raised plantlets in field environments. Different solutions were added to the substrates to study their effects on the number of roots and other botanical characteristics of rootless ginger tissue culture seedlings. Compared to substrate without solution added, 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 2). The absence of auxin in the substrate resulted in a larger stem diameter and the addition of 1.0 mg/L NAA resulted in more rooting. In general, the growth when water was used as solution 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 auxin 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 3). However, root weight was significantly greater in substrates with RH 80% 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 substrates 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 matrix. These antimicrobial agents were capable of inhibiting 100% of microorganism growth in rootless tissue culture seedlings and field seedlings. Data in Table 4 showed significant differences concerning the number at the level of 5% among the applied different treatments. These data also revealed a negative relationship between increasing sodium hypochlorite concentration and plant height, root number, fresh weight, and botanical characteristics (Table 4). Therefore, the bacteriostatic agent inhibited the growth of ginger seedlings in addition to inhibiting microorganisms. The maximum number of roots (4.000 ± 1.472 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 with a low reproduction coefficient (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 rhizomes fragments (Kasilingam et al. 2018; Abed et al. 2016). Therefore, in-vitro propagation using tissue culture could be a suitable alternative for the effective production of ginger and ginger seedlings that can eliminate the transmission of pathogens. This will reduce costs as the bulkiness of ginger rhizomes as planting material makes their handling costly and laborious. Furthermore, to improve asexual crop species, most efforts have been restricted to evaluating individuals and selecting for desired traits. However, ginger crops may be improved by clonal multiplication through the induction of multiple shoots, as has been reported by several studies (Jagadev et al. 2008; Mohanty et al. 2008). 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 this rapid propagation 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 in 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 were domesticated in the peatmoss + sand + vermiculite treatment, with a development 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* was 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 in-vitro rooted ginger plantlets in culture vessels 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).

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. The rooting effect was different when different nutrient solutions and auxin were added to the substrate. 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 auxins (Abbas et al. 2011; Mehaboob et al. 2019). Different types and concentrations of auxins have varying effects on in-vitro root induction in different Zingiberaceae species (Mehaboob et al. 2019; Jualang et al. 2015). Determining the optimum type and concentration of auxin 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. 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.

This study also sought to identify optimal relative humidity (RH) range 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. There were no significant differences in ginger seedling growth among different humidity conditions. Generally, high RH levels decrease transpiration and water loss, which supports the high turgor pressure necessary for cell expansion and growth in adventitious roots and prevents desiccation (Loach 1988). This may have been why the plant height and stem diameter of rootless ginger tissue culture seedlings were lowest in the 60% RH treatment. However, our results showed that 80% RH was adequate for rapid rooting, whereas higher levels of RH reduced the rooting success. 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), and lower water potentials actually had the highest rooting and lowest mortality (Tombesi et al. 2015). One ecological explanation could be that plants put resources into growing structures that enable them to obtain more resources when stressed. Under water stress, ginger tissue culture seedlings tended to invest more resources in root growth.

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). In conventional photomixotrophic micropropagation, the sugar-containing medium must be carefully washed off the plant before transplanting to ex-vitro conditions. In this study, rootless tissue culture seedlings were planted in vermiculite and peat supporting material, which were sugar free and had with almost no bacterial and fungal pollution. However, because of the semi-enclosed and high humidity environment, sometimes there was some microbial pollution. 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.

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 hetero 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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Tables

Table 1 Effect of different substrates 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
general vegetable seedling-raising substrates	7.730±	3.801±	4.933±	0.905±	1.359±	0.450±	2.617±	0.204±
Peat	1.255 b	0.448 a	1.569 c	0.135 cd	0.072 b	0.030 e	0.183 d	0.016 c
coconut bran	9.093±	3.997±	6.733±	1.199±	1.850±	1.491±	4.361±	0.321±
vermiculite	1.569 a	0.404 a	2.594 b	0.039 b	0.212 a	0.341 a	0.322 a	0.027 a
vermiculite:peat (1:1(v/v))	8.313±	3.813±	6.867±	1.019±	2.019±	0.978±	3.963±	0.248±
Perlite:vermiculite (1:1(v/v))	0.814 ab	0.378 a	2.029 b	0.089 c	0.260 a	0.153 c	0.111 b	0.008 b
	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
	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
	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 2 Effects of different solutions 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
H ₂ O	6.417±	3.399±	4.083±	0.834±	0.871±	0.381±	2.082±	0.163±
0.5MS	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+0.5 mg/L NAA	8.217±	3.324±	4.000±	1.122±	1.037±	0.319±	2.452±	0.179±
0.5MS+1.0 mg/L NAA	1.364 a	0.544 a	1.080 b	0.055 a	0.175 b	0.106 d	0.342 b	0.018 bc
MS+0.5mg/L NAA	7.508±	2.987±	2.667±	0.952±	0.900±	0.188±	2.038±	0.150±
MS+1.0 mg/L NAA	1.237 ab	0.444 b	1.106 c	0.137 b	0.185 b	0.052 e	0.256 c	0.017 d
	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
	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
	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 3 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±
70%	1.035 b	0.465 b	1.312 a	0.074 a	0.019 a	0.029 b	0.034 ab	0.005 a
80%	7.483±	3.305±	2.333±	0.689±	0.774±	0.156±	1.545±	0.123±
90%	1.053 b	0.248 a	0.745 a	0.052 b	0.228 a	0.062 b	0.220 b	0.011 a
	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
	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 4 Effects of different antimicrobial agents and concentrations 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
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
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
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	0.081 c	0.150 b	0.007 e	0.234 b	0.020
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
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
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
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
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
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

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.