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OPTIMIZING THE PROPAGATION TECHNIQUES FOR *GARDENIA JASMINOIDES* ELLIS

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Abstract

In Fujian China the harvest time for *Gardenia jasminoides* fruit is generally from October to November each year. The seeds should be timely placed in the shade area or kept in the refrigerator (4-5°C), which could improve the seedling height increment, but could not improve the germination rate. 500 ppm 3-indolebutyric acid (IBA) was selected as the suitable plant growth regulator (PRG) and concentration for *G. jasminoides* cutting propagation by comparison of different PRG concentrations. *G. jasminoides* cuttings showed early flowering performance in seedling stage, and the fruiting would be advanced after afforestation. *G. jasminoides* explants were difficult to disinfect during explant initiation. The mother plants for taking explants should be pretreated with pesticides for reducing the contamination rate. Dark culture was needed and 5 mg/L Vitamin C (VC) was added into the medium to reduce the browning during the explant initiation culture. The medium that Murashige and Skoog basal medium (MS)+1.0 mg/L 6-benzyladenine (BA)+0.1 mg/L 1-naphthylacetic acid (NAA)+50 mg/L VC and 30 g/L sugar were appended in the explant initiation medium. The average multiplication rate of subcultural medium MS+0.5 mg/L BA+0.1 mg/L NAA+5 mg/L Vitamin B_2 (VB_2) +50 mg/L VC was 6.25 times, the average shoot height was 3.83cm, and the shoot base had less calli and the shoots grew well. The rooting medium 1/2 MS+ 0.25 mg/L IBA supplemented with 20 mg/L sugar, the rooting rate achieved 100%, the average root length reached 4.46 cm and the average root number per plant reached 7.92 cm. The tissue-cultured seedlings were moved to the glass greenhouse for hardening, then washed the culture medium and transplanted to a greenhouse by covering the plastic film to keep humidity. 30 days after careful control of pests and diseases, the survival rate was more than 98%.

Key words: *Gardenia jasminoides* Ellis; Germination rate; Cutting propagation; Tissue culture; Explant; Multiplication; Rooting.

Introduction

Gardenia jasminoides Ellis belongs to genus *Gardenia* of family Rubiaceae. They are 0.3 to 3 meters height and grow in bushes or forests near hills, valleys, and riversides at the altitude of 10-1500 meters. It contains Gardenia yellow pigment which can be extracted and used as natural pigment for food additives. Gardenia flower is white, representing pure and beauty, which is widely planted as a landscaping tree species.

G. jasminoides has strong adaptability to the surroundings, less investment and quick and high economic benefit. It can be planted in all mountainous areas in southern provinces. In the middle and late of 1990s, Jiangxi, Sichuan, Guangxi, Hunan, Hubei, Henan and other main domestic provinces started to plant it. There was a lot of Gardenia cultivation in Fuding City, Fujian Province, covering more than 4,000 hectares, and the current total area in Fujian province is more than 6,667 hectares.

In recent years, with the rapid development of traditional Chinese medicine and ornamental flower industries, the wild as well as cultivated resources of *G. jasminoides* have been far from meeting the market demand. In order to meet the needs of production and breeding, it is necessary to carry out research on propagation techniques of *G.jasminoides*, including seed propagation, cutting propagation and tissue culture propagation. A preliminary summary and description of Gardenia seedling raising techniques were described, but the effects of storage methods and seeding methods on seed germination rate and seedling growth were not studied (Feng, 2018; Yang *et al*., 2018). As for the cutting propagation, the plant growth regulator (PRG) concentration was 800 mg/L indole-3-acetic acid (IAA), the cuttings were soaked for 2 hours, and the rooting rate reached 85.42% (Wang *et al*., 2020). *G. jasminoides* var. *grandiflora* cuttings were soaked in 300 mg/L 3-indolebutyric acid (IBA)+ 300 mg/L naphthaleneacetic acid (NAA) for 30 min, and *G. jasminoides* var. *radicans* cuttings were soaked in 500 mg/L IBA+500 mg/LNAA for 30 min, their rooting rate reached the highest with 95% and 98%, respectively (He & Yang, 2018). The tissue culture propagation of *G.jasminoides* has been reported, the subcultural multiplication rate was 2.95-4.63, and the rooting rate was 64.0-89.4% (Cui, 2012; Cui *et al*., 2012; Lu *et al*., 2000; Xia, 2007; Zhao *et al*., 2005). It is of great significance to improve seeding germination rate, cutting rooting rate, tissue culture multiplication rate and rooting rate of *G. jasminoides* for seedling growing and breeding.

Material and Methods

Overview of test site: The test site is located at the nursery of Fujian Academy of Forestry Sciences, Fuzhou city, Fujian Province. It belongs to the subtropical maritime monsoon climate, with warm climate and abundant rainfall. The annual average temperature is 20-25°C, the coldest month from January to February is 6-10°C, the hottest month from July to August is 33-37°C, the frost-free period is about 326 days and no snow, the annual average relative humidity is about 77%, and the annual average precipitation is 900-2100 mm.

Seeding propagation: *G. jasminoides*fruits were collected, sealed in the bags and stored in the dark of the laboratory or in a refrigerator (4-5°C). There were three storage methods: S1: cool storage on the day of picking, S2: room temperature storage for 1 week after picking

and then cool storage, S3: room temperature storage for 2 weeks after picking and then cool storage. Generally, the pericarp began to rot after 7 days, and most of the pericarp rotted after 14 days. Fruits were squeezed, crushed, placed in a stainless steel strainer and rinsed under tap running water until all debris was totally washed away. The seeds were then placed on paper in a cool, dry place and dried under a running fan, then sealed in a plastic bag and stored in a refrigerator (4-5°C).

The *G. jasminoides* seeds were collected from 5 sites, 900 seeds each sources 300 seeds per repeat, and 3 repeats. The *G. jasminoides*seeds were mixed with fine sand and evenly scattered on the red-core soil, then covered with disinfected river sand with a thickness of about 2 cm (the red-core soil and river sand was disinfected with 1/10000 potassium permanganate 3 days before sowing). Spring seeding was done in March, and winter seeding in November or December. The plastic frames with seeds were placed in the greenhouse and sprayed with water regularly in every 2-3 days after seeding. Immediately after seeding, 50% Carbendazim wettable powder or 70% Mancozebwettable powder 1000-times solution was thoroughly sprayed on the substrate to prevent seed diseases.

3-4 months after sowing, seedlings grew to more than 3cm height and could be transplanted into plastic containers with one plant percontainer, or into plastic frames of 40 cm length×30 cm width×10 cm height, with 35 plants in each frame. They were sprayed twice (once every half a month) with the disinfection solution mentioned above to prevent seedling diseases. After a month, 0.1-0.2% liquid compound fertilizer was applied.

Cutting propagation: Local red-core soil was used as cutting substrate. The 70% external shade covered over the greenhouse. Three days before cutting, the substrate was thoroughly disinfected with 0.01% potassium permanganate solution and covered with film until cutting.

*G. jasminoides*cutting scions were collected from mother trees of various ages. Cutting scions of 8-12 cm length with 2-4 leaves each were immersed in water immediately after cutting to prevent water loss. The cuttings were then immersed in a solution of 70% Mancozebwettable powder 1000-times dilution for 10-15 min. The base of the cuttings were treated with corresponding rooting agent (IBA; 0, 100, 500, 1000 or 2000 ppm), and then inserted into the substrate with depth of the 2/5-1/2 scion length. Arch was covered with a transparent plastic film to keep moist after cutting done. They were sprayed with water once a day on sunny days or once every 2-3 days on cloudy days and with a disinfection solution mentioned above every 10 days. After 30 days the transparent plastic film was removed, and the frequency of water spraying increased. The survival rate, number of roots and root length were evaluated 2 weeks after the film was taken off. A randomized block design was used with 3 repeats and 180 plants per treatment.

Tissue culture propagation: The apical buds or semi-lignified stems of *G.jasminoides* were used as explants. The explants were rinsed with running water for 5-10 min, the leaves were removed, the explants were treated with family liquid detergent dilution for 10-15 min, and then rinsed with purified water. In a laminar flow machine, the explants were sterilized in 70-75% alcohol for 60 s, followed with 0.1% $HgCl_2$ for 12-15min, and then rinsed with autoclaved water 4-5 times. The ends of stem segment were cut off, kept about 1cm length and incubated onto various explant initiation media (Tang, 1991).

Decontaminated explants were cultured in dark for 2 weeks, and then light intensity increased to 1000-1500 lux during subculture. Light intensity 1000-1500 lux was for rooting culture. Rooting culture was carried out in the culture room in the early stage, and the seedlings were moved into the greenhouse in the later stage for hardening by natural light, and the light intensity increased to 3000-6000 lux. The room temperature was controlled at 25±2°C and the illumination time was 12 hours per day.

Experimental design: The explant initiation media: I1: Murashige and Skoogbasal medium (MS)+1.0 mg/L 6-benzyladenine (BA)+0.1 mg/L NAA+50 mg/L Vitamin C (VC); I2: 1/2MS+1.0 mg/L BA+0.1 mg/L NAA+50 mg/L VC; I3: MS+0.5 mg/L BA+0.1 mg/LNAA+50 mg/L VC; I4: MS+0.5 mg/L BA+0.1 mg/L NAA+50 mg/L VC (Chen *et al*., 2014; Chen *et al*., 2016a; Chen *et al*., 2016b; Chen *et al*., 2017). The above media were supplemented with 30 g/L sugar and 6.0 g/L agar powder, pH6.0. Each treatment was 40 bottles, one bud or stem segment each bottle, and 3 repeats. The contamination and initiation were investigated after 30 days of culture.

Multiplication media: M1: MS+1.0mg/L BA+0.1 mg/L NAA+5 mg/L Vitamin B_2(VB_2)+50 mg/L VC; M2: MS+0.5 mg/L BA+0.1 mg/L NAA+5 mg/L VB2 +50 mg/L VC; M3: MS+ BA0.1+NAA 0.1+5 mg/L VB2 +50 mg/L VC. The above media was supplemented with 30 g/L sugar and 6.0 g/L agar powder, pH 6.0. Each treatment was 30 bottles, each bottle contained 5 stems or buds, and 3 repeats. After 30 days of culture, the multiplication rate and shoot height were calculated.

Rooting media: R1: 1/2MS+0.25 mg/L IBA; R2: 1/2MS+0.5 mg/L IBA; R3: 1/2MS+1.0 mg/L IBA. The above media were all supplemented with 20 mg/L sugar and 6.0 mg/L agar powder, pH6.0. Each treatment was 10 bottles, each bottle contained 8 shoots, and 3 repeats.

Seedling hardening: The rooted seedlings were incubated in the culture room for 25 days, and then transferred to the glass greenhouse for 15 days. Light intensity was 2000-6000 lux. The rooting rate, root number, root length and shoot height were investigated 40 days after rooting incubation.

Transplanting and management: The red-core soil was used as the transplanting substrate, and was put into a plastic frame, and moved into a greenhouse. The soil was disinfected with 0.01% potassium permanganate. The hardened *G. jasminoides* seedlings were washed with tap water, sank in a compound of 70% Thiophanate Methylwettable powder 1000-times dilution and 80% Mancozebwettable powder 1000-times dilution for 10 min for disinfection, then transplanted into plastic frames,

covered with film to keep moisture. After 2 weeks, the film was uncovered. Spray water 1-3 times per day.

Data analysis methods: Excel was used for Statistics, and SPSS Statistics 17.0 software was used for data one-way ANOVA with confidence of 0.01 or 0.05.

Results and analysis

Germination rate of seeds from different sources: The seeds were sown in March 2020, and the germination rate was calculated and analyzed in September 2020 (Table 1). The average germination rate of E1 (Fuding seeds) was 53.7%, which was significantly higher than E2 (Jian'ou seeds), E3 (Fuzhou seeds), E4 (Qingliu seeds) and E5 (Jiangxi seeds), and there were significant differences or highly significant difference among them. The average germination rate of E3 (Fuzhou seeds) and E4 (Qingliu seeds) was 34.4% and 34.2%, with no significant or highly significant difference between them, but both were significantly higher than E2 (Jian'ou seeds) and E5 (Jiangxi seeds), with significant or highly significant difference. The 3-month-old seedlings of Fuding seeds are shown in Fig. 1, and the 6-month-old seedlings of Fuding seeds are shown in Fig. 2.

Differences of seed germination rate and seedling height from different storage methods: The experimental *G. jasminoides* seeds were collected from Fuding City on October 21, 2019, and 1200 seeds were sampled from each source, 400 seeds per repeat and 3 repeats. The seeds were sown in March 2020, and the germination rate was calculated and analyzed in September 2021 (Table 2 and Fig. 3). There was no significant difference or highly significant difference in seed germination rate among three storage methods, S1, S2 and S3. There was significant difference in seedling height, and S1 was superior to S3, that is, the seedling of cool storage on the day of picking was higher than that of room temperature storage for 2 weeks after picking and then cool storage.

Effect of PRG concentration on cutting rooting: The effect of PRG concentration on cutting rooting of *G. jasminoides* was shown in Table 3. The PRG concentration had no significant or highly significant on the rooting rate and root length. PRG concentration had no significant difference in plantlet height, but there was significant difference between T3 treatment and T1 treatment (control, no PRG). The plantlet height of T3 treatment was 9.27 cm, which was significantly higher than that of the control T1 (7.96 cm). The number of lateral roots per plant in T5 treatment was significantly higher than that of T1 treatment (control, no PRG), and there was no significant difference between T5 treatment and other treatments. T5 treatment was significantly different from T1 treatment, T2 treatment and T4 treatment, but T5 treatment was not significantly different from T3 treatment. In conclusion, the effects of T5 treatment on rooting rate, plantlet height, lateral root length and lateral root number reached the highest level of A or a, and fine roots were induced. Therefore, 500 ppm IBA of T5 treatment was selected as the suitablePRG concentration for *G. jasminoides* cuttings. Rooting results of each treatment were shown in Figs. 4 and 5 shows the early flowering phenomenon of *G. jasminoides* in plantlet stage after cutting.

Aseptic culture of seeds in tissue culture: For sterile seeding of *G. jasminoides*, MS+30g/L sugar medium without PRG was applied. Rooting effect was shown in Fig. 6.

Selection of culture medium for explant initiation: The apical buds or semi-lignified twigs of *G.jasminoides* were used as explants. The results of *G. jasminoides* explant initiation were shown in Table 4. It was found that the explants were susceptible to browning, so 5mg/L VC was added to reduce explant browning. Table 4 showed that medium T1 had the highest average initiation rate, reaching 58.33%, which was significantly different from the medium T2, T3 and T4, but the calli were more and the initiated buds were more and smaller.

Table 1. Analysis of *G. jasminoides* seed germination rate of different sources.

Test code	Source	Total seed number	Germination number	Germination rate /%
E1	Fuding	900	483	53.7 ± 1.30 Aa
E2	Jian'ou	900	217	24.1 ± 0.75 Cd
E3	Fuzhou	900	310	34.4 ± 0.61 Bb
E4	Qingliu	900	308	34.2 ± 0.17 Bb
E5	Jiangxi	900	246	27.3 ± 1.25 Cc
Average				34.7

NB: 1. Same capital letter in same row means no highly significant difference, different capital letter in same row means highly significant difference, and A>B>C, $\rho<0.01$;2. Same lowercase letter in same row means no significant difference; different lowercase letter in same row means significant difference and a>b>c>d, $\rho<0.05$

Table 2. Analysis of *G. jasminoides* seed germination rate and seedling height of different storage methods.

Test code	Source	Storage	Germination rate /%	Seedling height/cm
S1	Fuding	S1	52.5±3.32 Aa	6.7±0.45 Aab
S2	Fuding	S2	57.2±4.40 Aa	6.9±0.42 Aa
S3	Fuding	S3	53.1±2.94 Aa	5.6±0.07 Ab
Average			54.2	6.4

NB: 1. Same capital letter in same row means no highly significant difference, $\rho<0.01$; 2. Same lowercase letter in same row means no significant difference; different lowercase letter in same row means significant difference and a>b, $\rho<0.05$

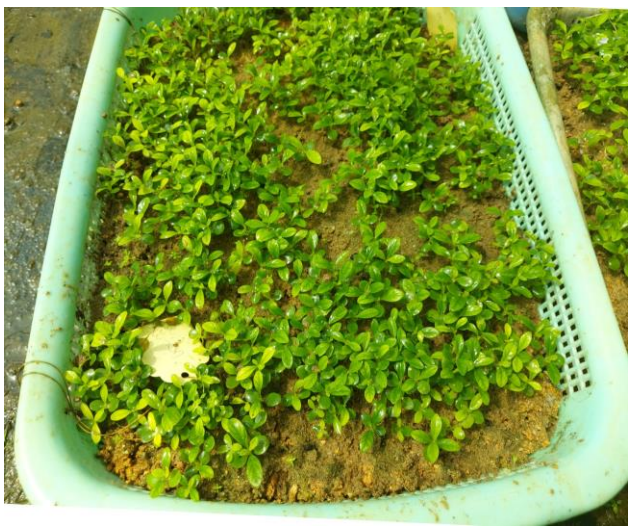

Fig. 1. Three months after seeding of *G. jasminoides*.

Fig. 2. Six months after seeding of *G. jasminoides*.

Fig. 3. Fruit color changes of *G. jasminoides*from cool storage from the day of picking and storage at room temperature (post-ripening phenomenon)
Left: cool storage from the day of picking; Right: storage at room temperature for 16 days

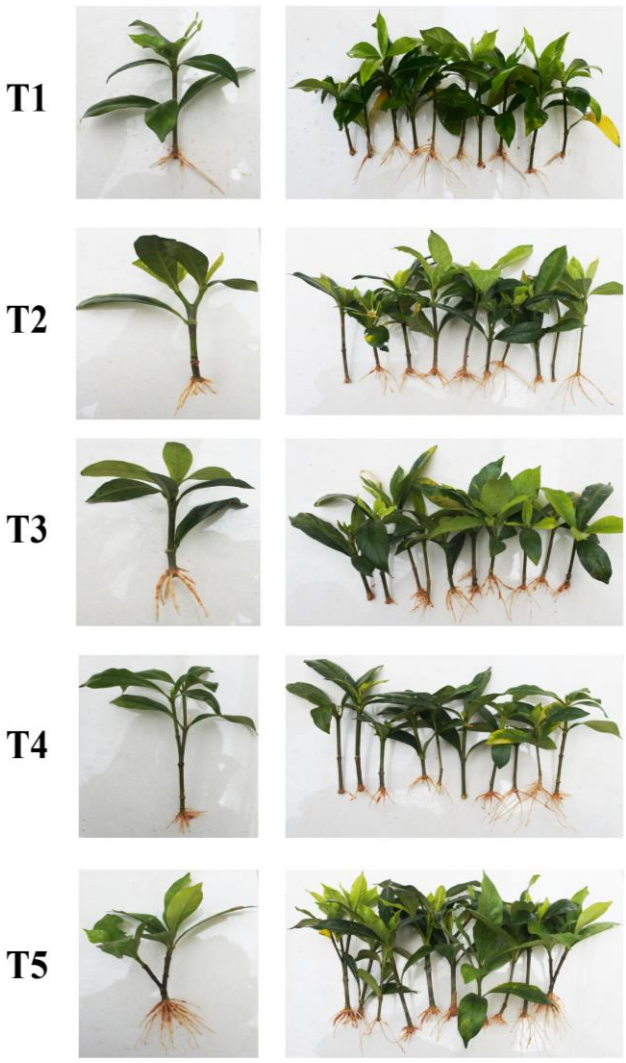

Fig. 4. Rooting effects of each treatment in *G. jasminoides*.

Fig. 5. Early flowering phenomenon of *G. jasminoides* in seedling stage.

Selection of multiplication media in tissue culture: In the laminar flow machine, the new shoots were cut off and transferred to the multiplication medium. The results were shown in Table 5. The analysis results showed that the average multiplication rate of medium M1 was 9.43 times, which was significantly different from that of medium M2and M3. However, due to the abundance of calli, small buds and vitrified leaves at the base of shoots, M1 was not suitable for subculture. The average multiplication rate of medium M2 was 6.25 times, which was significantly different from that of medium M3. The average shoot height was 3.83 cm, which was the best than that of medium M3, the calli at the base of shoots in medium M2 was little and the shoots grew vigorously. The average multiplication rate of medium M3 was only 2.74, so medium M2 was selected as the optimal multiplication medium for *G. jasminoides* (Fig. 7).

Selection of rooting medium in tissue culture: The shoots of *G. jasminoides* obtained by multiplication culture were transferred to the medium 1/2MS, supplemented with IBA of different concentrations. The seedlings were cultured in a culture room for 25 days, and then transferred to a glass greenhouse for 15-days' hardening. The rooting indexes were invested, and the analysis results were shown in Table 6. The average rooting rate of R1, R2 and R3 all reached 100%, and there was no significant difference or highly significant difference among them. There were no highly significant differences in average root length, average root number per plant and average seedling height among Medium R1, R2 and R3. The average root length of medium R1 was 4.46 cm, not significantly different from that of medium R2, but significantly different from that of medium R3 (3.15 cm), and R1 and R2 were superior to R3. The average root number per plant in medium R1 was 7.92 cm, which was significantly different from that of medium R2 and R2, and R2 and R3 were superior to R1. There was no significant difference in average seedling height among the three media. Taking the factors above into consideration, the average number roots per plant of medium R1 reached 7.92 cm, which had achieved the level of industrialized seedling production, moreover, considering the cost, medium R1 contained the lowest concentration of IBA, therefore, medium R1 was selected as the optimal rooting medium for *G. jasminoides* (Fig. 8).

Transplanting of tissue-cultured seedlings: After 15 days of hardening, the tissue-cultured seedlings of *G. jasminoides* were transplanted to a greenhouse after the medium washed. The substrate was local red soil, which was sterilized by 0.03%-0.05% potassium permanganate. After transplanted into thesubstrate, the seedlings were covered with thin plastic film to keep moisture and control pests and diseases. No phenotypic variation was observed in seedlings (Figs. 9 and 10).

Table 3. Effects of PRG on cutting rooting of *G. jasminoides*.

Test code	IBA concentration /ppm	Average rooting rate /%	Average seedling height/cm	Average lateral lenght/cm	Average root number per plant/strip	NB
T1	0	99.7 ± 0.33Aa	7.96 ± 0.549Ab	1.50 ± 0.372Aa	5.92 ± 0.507Bb	No fine root
T2	100	99.7 ± 0.33Aa	9.27 ± 0.799Aab	1.52 ± 0.176Aa	6.36 ± 0.811ABb	Fine root
T3	500	99.3 ± 0.67Aa	9.67 ± 0.209Aa	1.68 ± 0.214Aa	8.22 ± 1.060ABab	Fine root
T4	1000	95.0 ± 4.04Aa	8.65 ± 0.178Aab	1.76 ± 0.420Aa	7.44 ± 1.160ABb	Fine root
T5	2000	97.3 ± 2.19Aa	8.52 ± 0.173Aab	1.97 ± 0.137Aa	10.78 ± 1.176Aa	Fine root
Average		98.2	8.81	1.68	7.74	

NB: 1. Same capital letter in same row means no highly significant difference, different capital letter in same row means highly significant difference, and A>B>C, ρ<0.01;2. Same lowercase letter in same row means no significant difference; different lowercase letter in same row means significant difference and a>b>c>d, ρ<0.05

Table 4. Effect of *G. jasminoides* explant initiation medium.

Medium code	Basal medium	BA/mg/L	NAA/mg/L	Average initiation rate/%	Growth performance
I1	MS	1.0	0.1	58.33±2.324 Aa	More calli, more shoots, and smaller shoots
I2	1/2 MS	1.0	0.1	40.74±1.485 Cc	More calli, less shoots, and smaller shoots
I3	MS	0.5	0.1	48.15±0.646 Bb	Less calli, and vigorous shoots.
I4	1/2 MS	0.5	0.1	34.88±0.284 Cd	No callus, less shoots, and smaller shoots
Average				45.53	

NB: 1. Same capital letter in same row means no highly significant difference, different capital letter in same row means highly significant difference, and A>B>C, ρ<0.01; 2. Same lowercase letter in same row means no significant difference; different lowercase letter in same row means significant difference and a>b>c>d, ρ<0.05

Table 5. Effect of *G. jasminoides* multiplication medium.

Medium code	Medium	BA/mg/L	NAA/mg/L	Average multiplication rate/times	Average shoot height/cm	Growth performance
M1	MS	1.0	0.1	9.43 ± 0.225Aa	2.67 ± 0.095Bc	More calli, more and smaller shoots, and vitrification leaves
M2	MS	0.5	0.1	6.25 ± 0.093Bb	3.83 ± 0.070Ab	Less calli, and vigorous shoots
M3	MS	0.1	0.1	2.74 ± 0.116Cc	4.24 ± 0.067Aa	No calli, and less vigorous shoots
Average				6.14	3.58	

NB: 1. Same capital letter in same row means no highly significant difference, different capital letter in same row means highly significant difference, and A>B>C, ρ<0.01; 2. Same lowercase letter in same row means no significant difference; different lowercase letter in same row means significant difference and a>b>c, ρ<0.05

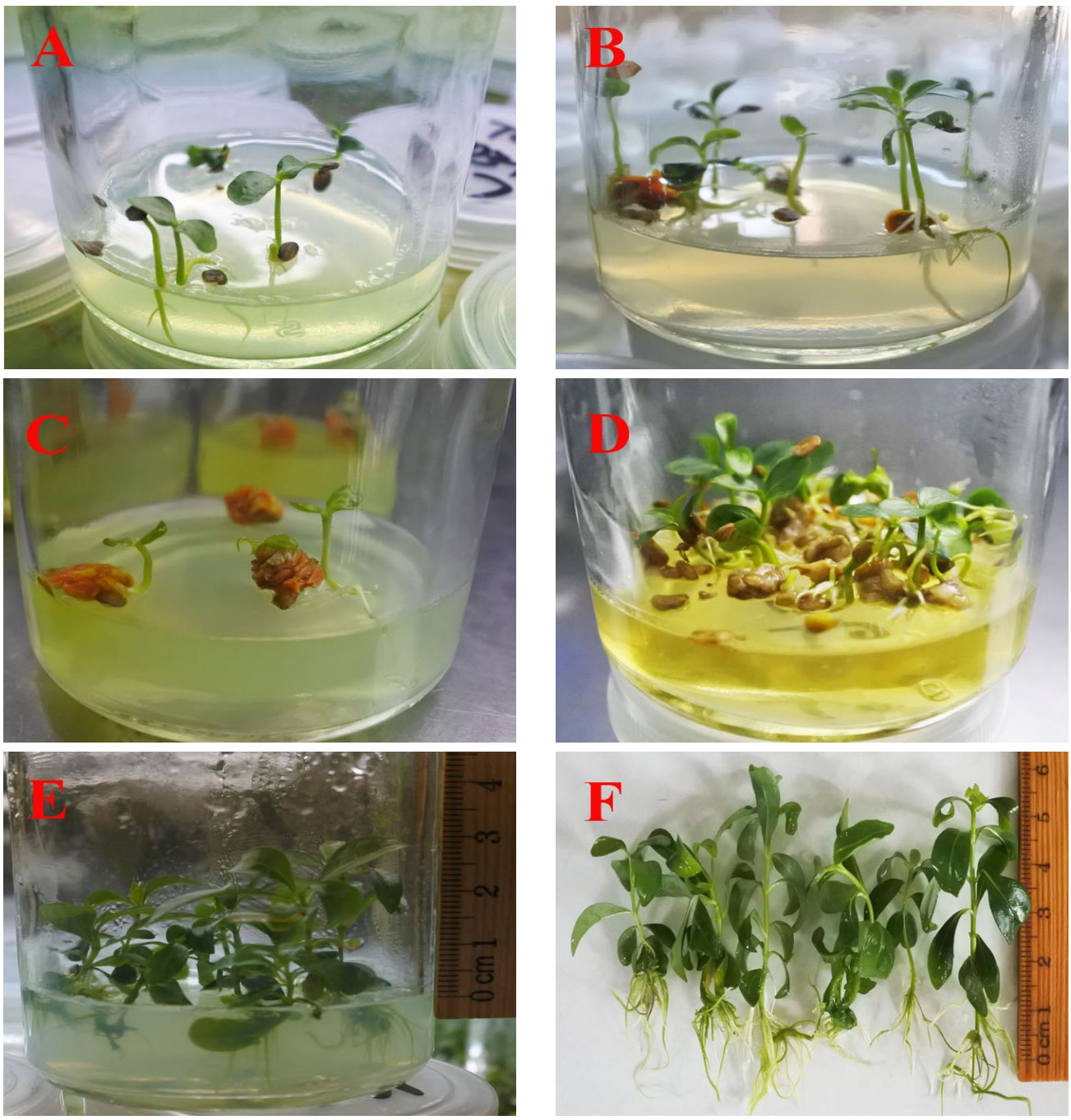

Fig. 6. Growth of *G. jasminoides*aseptic seeding
Note: A and B were pure seeds 2 months after culture; C and D were seedlings with berry contents 2 months after culture; E and F were seedlings of 4-month culture.

Table 6. Effect of *G. jasminoides* rooting medium.

Medium code	Medium	IBA/ mg/L	Average rooting rate /%	Average root lenght/ cm	Average root number per plant /stripe	Average seedling height/cm	Growth performance
R1	1/2MS	0.25	100.00 ± 0.000Aa	4.46 ± 0.449Aa	7.92 ± 0.333Ab	3.20±0.208Aa	Vigorous growth seedlings
R2	1/2MS	0.50	100.00 ± 0.000Aa	3.56 ± 0.050Aab	9.50 ± 0.866Aa	3.03±0.296Aa	Vigorous growth seedlings
R3	1/2MS	1.00	100.00 ± 0.000Aa	3.15 ± 0.230Ab	9.75 ± 0.000Aa	2.67±0.219Aa	Vigorous growth seedlings
Average			100.00	3.72	9.06	2.97	

NB: 1. Same capital letter in same row means no highly significant difference, ρ<0.01; 2. Same lowercase letter in same row means no significant difference; different lowercase letter in same row means significant difference and a>b, ρ<0.05

Fig. 7. Growth performance of *G. jasminoides* multiplication materials
Note: Left: 2 bottles with M1; Right: 2 M2 bottles with M2

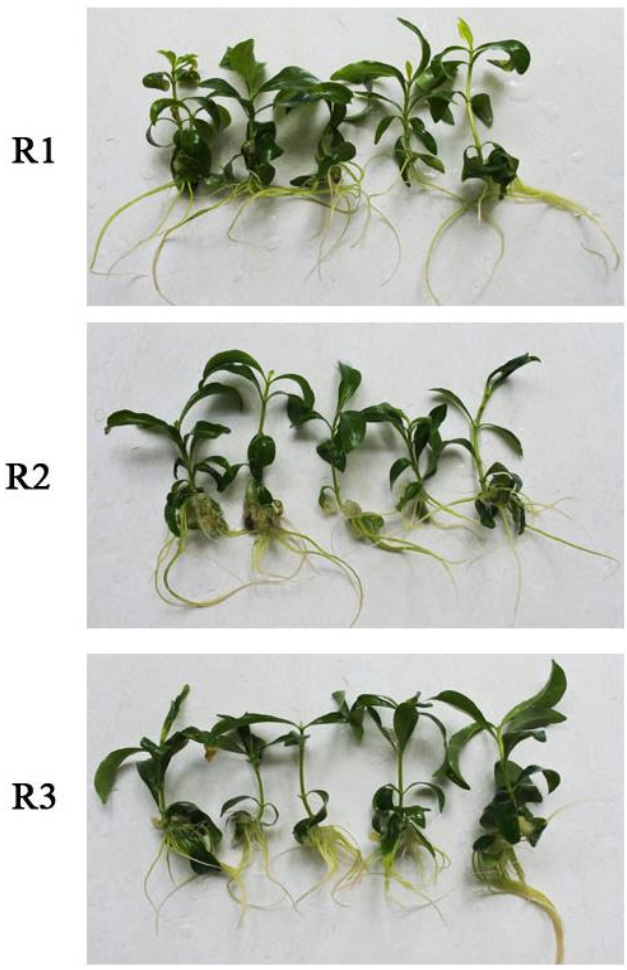

Fig. 8.Growth performance of *G. jasminoides* rooting materials.

Fig. 9. Tissue-cultured seedlings of *G. jasminoides*hardening in a greenhouse.

Fig. 10. Tissue-cultured seedlings of *G. jasminoides* survived in a greenhouse after transplanted 20 days.

Conclusions and Discussion

The harvest time of *G. jasminoides* fruits in Fujian is from October to November every year. They should be placed in the shade or refrigerated (4-5°C) in time. They should not be exposed to strong sunlight or steamed and dried, otherwise the seeds cannot germinate. There was significant difference or highly significant difference on the seed germination rate in greenhouse from different sources. The seeds from Fuding had the highest germination rate, with an average germination rate of 53.7%. The three storage methods had no significant or highly significant difference on seed germination rate, but had significant difference in seedling height, that is, the seedling of cool storage on the day after picking was higher than that of room temperature storage for 2 weeks after picking and then cool storage. It is recommended that *G. jasminoides* fruits should be refrigerated (4-5°C) on the day after harvesting.

G. jasminoides are easier to root. Through the comparison test of different concentrations of PRG, 500 ppm IBA was selected as the suitable concentration of PRG for *G. jasminoides* cuttings. After 2 months of cutting, the average rooting rate reached 99.3%, the average seedling height reached 9.67 cm, the average lateral root length reached 1.68 cm, and the average number of lateral roots per plant reached 8.22. There were fine roots on the lateral roots. The cuttings showed early flowering phenomenon at seedling stage, which could brought forward the fruit after afforestation.

G. jasminoides explants were difficult to disinfect, and pretreatment of the stock plants could reduce the contamination rate. Spray the mother plants with a mixture of 4.5% efficient Cypermethrin emulsion 1000-times dilutionand 5% Imidacloprid emulsion 800-times dilution, and mixed with 70% Methyl Topzinwettable powder 800-times dilution or 80% Mancozebwettable powder 800 times the next day, the explants were collected on clear days within one week after spraying, and the average contamination rate of explants decreased from 81.25% to 40.40%.

It was found that the explants were easy to browning during the initiation incubation. 5 mg/L VC was supplemented and incubated in dark to reduce browning.

The results showed that medium MS+1.0 mg/L BA+0.1 mg/L NAA+50 mg/L VC supplemented with 30 g/L sugar was the optimal for explant initiation, and the initiation rate was the highest, up to 58.33%, which was significant difference from other media, but the calli were more on the base of explants and the induced buds were more and smaller. The subculture medium must be transferred in time to prevent excessive callus growth. The results were consistent with those of tissue culture test of *Dendrobium officinale* and eucalypts (Cao & Stephen, 2012; Chen *et al*., 2006; Chen *et al*., 2014). The average multiplication rate of MS+0.5 mg/L BA+0.1 mg/L NAA +5 mg/L VB2 +50 mg/L VC was 6.25, the average shoot height was 3.83 cm, the base of shoots had vigorous buds and less calli, and this medium could be used as the multiplication medium of *G. jasminoides*.

With the addition of three different concentrations of IBA in 1/2MS medium, the rooting rate in the bottle reached 100%, indicating that *G. jasminoides* was easy to root. Considering the cost of propagation, medium 1/2 MS+0.25 mg/L IBA, supplemented with 20 g/L sugar, the average root length reached 4.46 cm, the average root number per plant reached 7.92, and this medium could be used as the rooting medium for tissue culture of *G. jasminoides*.

The tissue-cultured seedlings were hardened, washed and transplanted to a simple greenhouse, covered with plastic film to keep moisture and to control of pests and diseases, the survival rate reached more than 98% after 30 days.

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