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The Influence of Different Culturing Condition on the Regeneration Induction of Explant Stem

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Article

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Abstract

Background: The tissue culturing is one the most rapid methods to fasten the reproduction and to enlarge the cultivation scale. However, during the culture process, the browning and pollutionof explant is the most important reason for the failure. Therefore, the browning and pollutionprevention is very critical. This article studies the browning, pollution and sprouting of explants of three species under different temperature and different sucrose concentration to identify the influence of a single factor on the regeneration induction of explant stems.

Results: The experiment result shows that in 10℃ the browning and pollution are both inhibited. Meanwhile the low temperature also led to a low sprouting rate of explant. In order not to affect the normal induction of differentiation, we should reduce the culture temperature appropriately. Under the appropriate amount of sucrose concentration (20-25g/L), pollution rate and sprouting rate showed positive effect except for the slight increase of browning.

Conclusions: These results can provide a theoretical basis for the establishment of technical standards for the industrialized production of good quality seedlings of *Euonymus japonzcus thumb* and *Taxus chinensis* and *Ficus carica* L.

Key message

The optimal culture conditions identified from explant trials on three plant species offer both theoretical insights and practical reference value for the industrial-scale production of high-quality seedlings.

Introduction

Euonymus japonicus Thunb belongs to the family Celastraceae. It is native to the South of China and is common because of ornamental and medical properties. People often collect its stem skin and branches and leaves, dry them in the sun and reserve them for later use. According to Chinese Materia Medica, it is bitter, pungent and warm in nature, with an effect of eliminating wind-warm syndrome, strengthening muscles and bones, promoting the blood circulation and arresting the blood (State Administration of Traditional Chinese Medicine. 1999). It can mainly cure rheumatic arthralgia, soreness and weakness of waist and knees, wounded swollen, fracture and hematemesis. In recent years, with the increasing demand for the quantity and quality of *Euonymus japonzcus thumb* seedlings, the shortcomings of the traditional asexual reproduction (cutting propagation) have become increasingly prominent, and the breeding coefficient is low, which is difficult to meet the needs of production. And leaf spot disease is caused by the cutting propagation in the plum rain season. The propagation by tissue culturing can offer a large amount of good quality plant without any pathogens within a short time (Wu Guangyu et al.2005; Ma Shaomei.2020; Xue Huiwen et al.2015). And it can enlarge the propagation by batch and by period to relieve the tension of supply and demand and to guarantee the annual supply of the plants. The establishment of vegetative propagation system of *Euonymus japonzcus thunb* through tissue culturing is an effective way to obtain high quality and large quantity of plants.

Taxus chinensis, also named yew, is a plant of taxus and ancient precious tree species near extinction. The Taxol, a cancer-fighting drug made of paclitaxel which is extracted from *Taxus chinensis* is a most commonly used drug for lung cancer, ovarian cancer and breast cancer, and it has an obvious effect on malignant melanoma, tumor on the head and neck, leukemia and colon cancer (Yang Lixiang et al.2001;Xia Hongping.2005; Fu Zhetun et al.2006;Sun S M et al.2024;Wu Shilonget al.2024). *Taxus chinensis* grows very slowly under natural conditions and has a bad regeneration capacity. There is no big scale *taxus* raw material forest in the world up till now. The wild resource is very limited and it can hardly meet the needs of domestic and international market. Therefore, the artificial reproduction method is one the most effective solutions to the problem of resource shortage and the acceleration of *Taxus chinensis* forest building (Li Jinghong et al.2012). Tissue culturing can avoid the shortage of slow reproduction and to bring on the demand of the market. *Taxus chinensis* tissue culture has many advantages, such as abilitiy to be human control, short growth cycle, high reproduction rate, easy management and automated operation. (Chen Yongqin et al.2000; Sheng Changzhong et al.2000;Zhang Wenquan et al.2021; Yang Shushen et al.2024).

Ficus carica L. is a deciduous shrub or dungarunga of Ficus of Moraceae. Its fruit has a thin skin and no core, soft and sweet flesh and the editable rate is over 90%. The fruit contains a high rate of fructose, tartaric acid, protein and vitamins and has a very high nutrition value. According to the modern pharmacological study, it also has a medical value of invigorating stomach, purge, resolving toxin and dispersing swelling, anti-tumor, lowering the blood tension, hypocatharsis, lactogenesis, and assisting digestion (S Mawa et al.2013; Zhang Qianqian et al.2025). *Ficus carica* L. are widely distributed in the Mediterranean region, the Middle East, and desert areas of Egypt. However, they suffer from low propagation success rates and are highly susceptible to viral infections. However, during the culture process, the browning of explant is the most important reason for the failure. Therefore, the browning prevention is very critical (Zhou Junhui et al.2000; Zhu Jianhua et al.2002; Aboelhasan O M F et al.2025).

From the reported literature, there are few experiments on the optimum culture conditions for the tissue culture of *Euonymus japonzcus thumb*, *Taxus chinensis* and *Ficus carica* L., and there is no universally applicable culture method. This studies the browning, pollution and sprouting of explants of three species under different temperature and different sucrose concentration to identify the influence of a single factor on the regeneration induction of explant stems. The optimum conditions were explored and screened for the explants of three species in next step, and finally provide some reference in vitro culture and fast and high quality breeding of *Euonymus japonzcus thumb*, *Taxus chinensis* and *Ficus carica* L. in the future.

Materials and methods

Plant material

This study was conducted at the Tissue Culture Laboratory of Hubei Provincial Key Laboratory of Purification and Application of Plant Anti-cancer Ingredients. All plant materials used in this study were

cultivated, and the plant samples were obtained from the campus of Hubei University of Education. After cutting leaves with petiole remained and washing with tap waer the stems were cut into 5cm length pieces of explants. Then the explants were put in a sterile bottle with 75% ethylalcohol to soak them and shaken the bottle for 30 sec. When the alcohol was poured out, the explants were disinfected with mercuric chloride solution. Then the explants were washed with sterile water 5 times for 5 mins. The explants stem of each of the species was placed 4 in per bottle and it should be operated in 10 bottles for each treatment.Each Treatment was repeated 3 times.There is 120 explants stem of each species need to be prepared.

Preparation of culture medium

Initiating medium composition was based on MS (Murashige and Skoog 1962) medium, supplemented with 6-BA(6-Benzylaminopurine) 1mg/L + NAA (1-Naphthylacetic acid) 0.2mg/L, containing also 20g sucrose and 10g agar to make solid culture medium. Each round culture bottle was filled with 30 mL of culture medium and prepared for future use after sterilization.

Experiment processing

The experimental scheme was designed with single factor multi-level contrast design. Three temperature gradients were set at different temperature comparison tests: 10 ℃, 18 ℃ and 25 ℃. The sucrose concentration gradients were set at different concentration comparison tests: 15 g / L, 20 g / L and 25 g / L. Repeat 3 times per treatment. Other nutrients, growth regulators and culture conditions were the same, the explants of three species cultured on the medium, placed in light 12 h / d, light intensity 1 500-2 000 lx thermostats. The test results were observed and statistical after 3 days of culture.

Testing index

Browning rate= (the number of explants with browning phenomena/the total number of inoculated explants) ×100%.

Classification was carried out according to the browning grading standard proposed by Huang Hao and Li Jinghong(Huang Hao et al.1999; Li Jinghong et al.2016). Browning degree: Grade 0: without or small degree browning, the color of the culture medium around the material is milk white;Grade I：paint brown；Grade II：serious browning, brown; Grade III：serious browning, black brown.

Pollution rate=(the number of explants with pollution phenomena/ the total number of the inoculated explants)×100%.

Sprouting rate=(the number of explants with sprouting phenomena/the total number of inoculated explants)×100%.

Statistical analysis

Experiments data were analyzed by analysis of variance (ANOVA) using Statistical Analysis system (SAS) for single-factor randomized block test and tested for significance by Duncan's multiple range test (D.B. Duncan,1955).

Results

The influence of different temperature on explants

The influence of different temperatures on the browning of explants

As shown in Fig. 1-A, B and C, under different temperature treatment conditions, *Euonymus japonzcus thumb*, *Taxus chinensis* and *Ficus carica* L. explants have browning phenomenon occurs, the most serious is the *Ficus carica* L. (browning rate up to 90%), Followed by *Euonymus japonzcus thumb*, *Taxus chinensis* slighter (browning rate not more than 24%). With the increase of temperature, the browning rate of the explants of three species showed an increasing trend, and the *Ficus carica* L. were the most obvious (the difference was significant at different temperature treatments ($P < 0.05$)), while the increase of *Taxus chinensis* was not significant. As shown in Fig. 1-D, at the same temperature, the Browning rate of different varieties have reached significant level ($P < 0.05$), among them, browning rate of *Ficus carica* L. compared with *Euonymus japonzcus thumb* and *Taxus chinensis* the difference reached extremely significant level ($P < 0.01$).

Figure 1 **The Influence of Different Temperatures on the Browning of Explant**

From the browning grades (Table 1) it can be seen that the browning degree of *Euonymus japonzcus thumb* and *Ficus carica* L. increases with the rising of temperature. Under 25℃, the browning degree of most *Euonymus japonzcus thumb* stems is Grade II, and the color is brown; the browning degree of 50.1% *Ficus carica* L. stems is Grade III, and the color is black brown; while *Taxus chinensis* has a small variation, whose browning degree is Grade I under three different temperatures, and the percentage of browning is 16.2%, 21.2% and 23.2%, the color is light brown.

Table 1
The Percentage of Browning Grade of Explant under Different Temperatures (%)

Explant	Browning Degree	Temperature		
		10℃	18℃	25℃
Euonymus japonzcus thumb	Grade 0	77.8 ± 3.01	74.5 ± 2.51	54.0 ± 2.26
	Grade I	22.2 ± 2.72	17.9 ± 1.76	17.0 ± 1.81
	Grade II	0	7.6 ± 1.65	25.9 ± 1.67
	Grade III	0	0	3.1 ± 1.51
Taxus chinensis	Grade 0	83.8 ± 3.08	78.8 ± 3.65	76.8 ± 2.36
	Grade I	16.2 ± 2.04	21.2 ± 2.42	23.2 ± 2.23
	Grade II	0	0	0
	Grade III	0	0	0
Ficus carica L.	Grade 0	44.6 ± 2.88	27.7 ± 2.25	10.2 ± 1.86
	Grade I	50.0 ± 2.88	16.0 ± 1.93	13.5 ± 1.91
	Grade II	5.4 ± 1.80	30.2 ± 2.14	26.2 ± 1.84
	Grade III	0	26.1 ± 2.1	50.1 ± 2.59
Notes: Data are the mean ± SD of each group. The same as in the tables below.				

Table 1 **The Percentage of Browning Grade of Explant under Different Temperatures (%)**

The influence of different temperatures on the pollution of explants

Under the temperature of 10℃, 18℃ and 25℃, the explants of three species all have pollution phenomena, and with the rise of temperature, the pollution rate augment rapidly(Fig. 2-A,B and C). Under low temperature, the pollution rate of the three species is low, not higher than 15%; under high temperature, there are a lot of activities of micro-organisms, so they have a large pollution rate. Significant difference test showed that the same species, between the different temperature treatment are significant ($P < 0.05$); at the same temperature, pollution rate most significantly between different species ($P < 0.05$).

Figure 2 **The Influence of Different Temperature on Pollution of Explants**

Influence of different temperature on the sprouting of Explants

Under different temperature conditions, the sprouting rate of the explants of three species increased with the increase of temperature. The sprouting rate is very low at 10℃ and increases rapidly at 18℃, the highest sprouting rate at 25℃ (Fig. 3- A, B and C). Significant difference test showed that the same species, between the different temperature treatment are significant ($P < 0.05$); Only between *euonymus japonzcus thumb* and *ficus carica* L. there was no significant difference at 25℃, and the sprouting rate of the different species was significant ($P < 0.05$) at the same temperature.

Figure 3 **The Influence of Different Temperature on Sprouting of Explants**

The influence of different sucrose concentration on explants

The influence of different sucrose concentration on browning of explants

The results showed that the explants of three species were cultured at different sucrose concentration, with the increase of sucrose concentration, the browning rate of *Euonymus japonzcus thumb* and *Ficus carica* L. were increased, especially the browning rate of *Ficus carica* L. was between 80% and 90%; the browning rate of *Euonymus japonzcus thumb* is about 45%. the browning rate of *Taxus chinensis is* lower, which is about 23% (Fig. 4-A, B, C). Significant difference test showed that the browning rate of *Ficus carica* L. was significant between the different concentration treatment ($P < 0.05$); As shown in Fig. 4-D, under the same sucrose concentration, the browning rate between different species were significant ($P < 0.05$) and extremely significant ($P < 0.01$).

Figure 4 **The Influence of sucrose Concentration on Browning of Explants**

As can be seen from Table 2, the degree of browning of *Taxus chinensis* is relatively light, which are Grade I under three sucrose concentration with light brown; *Euonymus japonzcus thumb* browning degree are Grade I and Grade II; *Ficus carica* L. browning degree is higher, mostly are Grade III and Grade II with dark brown or black brown color. The browning degree of explants of all the three species increase with the increased concentration of sucrose.

Table 2
The Percentage of Browning Grade of Explants under Different Sucrose Concentration (%)

Explant	Browning degree	Sucrose concentration		
		15g/L	20g/L	25g/L
Euonymus japonzcus thumb	Grade 0	56.9 ± 2.82	53.2 ± 2.20	51.8 ± 2.80
	Grade I	43.1 ± 1.97	26.1 ± 1.59	16.1 ± 1.75
	Grade II	0	20.7 ± 2.15	30.1 ± 1.78
	Grade III	0	0	2.0 ± 0.56
Taxus chinensis	Grade 0	76.9 ± 2.55	77.1 ± 1.95	75.3 ± 2.06
	Grade I	23.1 ± 1.40	22.9 ± 1.95	24.7 ± 1.64
	Grade II	0	0	0
	Grade III	0	0	0
Ficus carica L.	Grade 0	16.2 ± 1.78	11.6 ± 1.66	8.1 ± 0.78
	Grade I	20.4 ± 1.85	16 ± 2.01	11.0 ± 1.12
	Grade II	25.6 ± 1.93	32.2 ± 1.90	28.3 ± 1.70
	Grade III	37.8 ± 2.35	40.2 ± 2.42	52.6 ± 2.46

Table 2 **Percentage of Browning Grade of Explants under Different sucrose Concentration (%)**

The influence of different sucrose concentration on the pollution of explants

As shown in Fig. 5-A, B and C, the pollution rate of explants of the three species are mostly between 20% and 30% under different sucrose concentration, and *Taxus chinensis* and *Ficus carica* L. did not change much. Only *Euonymus japonzcus thumb's* pollution rate rise at first and decline later with the rising of sucrose concentration. Significant difference test showed that the same species, mostly the pollution rate has no significantly between the different concentration treatment ($P < 0.05$); As shown in Fig. 5-D, under the same concentration of sucrose, the pollution rate between different species was only partly significant ($P < 0.05$).

Figure 5 **The Influence of sucrose Concentration on Pollution Rate of Explants**

The influence of different sucrose concentration on sprouting rate of explants

The results showed that the sprouting rate of explants of the three species was different, and *Taxus chinensis* was relatively low. The sprouting rate of explants of the three species did not change

significantly with the increase of sucrose concentration. Significant difference test showed that the same species had no significant difference between the different sucrose concentration ($P < 0.05$) (Fig. 6-A, B and C); Under the same sucrose concentration (Fig. 6-D), the sprouting rate between different species was significant ($P < 0.05$). In addition, half of the treatments were reached extremely significant level ($P < 0.01$).

Figure 6 **The Influence of sucrose Concentration on Sprouting Rate of Explants**

Conclusions and Discussion

The results showed that the browning rate, pollution rate and sprouting rate of *Euonymus japonzcus thunb, Taxus chinensis* and *Ficus carica* L. tended to increase with the increase of temperature in tissue culture process; At the same temperature, the response of different explants to the temperature was also different, and the difference among the different species was significant. Under the relatively low temperature conditions, the occurrence of browning and pollution phenomena have been inhibited, the degree of browning is also lighter. This basically complies with the previous study result, that is the temperature is too high to promote the biological activity of Polyphenol Oxidase (PPO), thereby promoting the oxidation of phenolic compounds to accelerate the browning of explants (Scondah IM M R et al.1981;González-Arnao, M. T. et al.2021; Rai, M. K. et al.2023; Han Shengnan et al.2023; Pan Xinhong 2025). Low temperature can inhibit the synthesis of phenolic compounds, thereby reducing the activity of Polyphenol Oxidase, reduce phenolic oxidation, thereby reducing browning (Hildebrandt V et al.1988;Patel, R. et al.2020; Gao, Y. et al.2023; Kumar, V.et al.2021).This shows that low temperature can inhibit the browning of plants. However, the low temperature (at 10℃) also caused a low sprouting rate of explants, Therefore, the temperature should be lowered to an appropriate degree in order to inhibit the browning and pollution of explants without influencing their normal induction and differentiation. In the future, we will conduct our experiments under different gradient so that we can find a most appropriate temperature; besides, a further study should be carried out for the application on different species even for a same temperature.

Sucrose is an important carbon source in tissue culture, and it is also the energy source of cell growth and the main components of the cytoskeleton. The amount of sucrose is one of the keys to the success of tissue culture. In this experiment, different concentrations of sucrose on browning rate, the degree of browning, pollution rate and sprouting rate of explants were affected. In addition to browning increased, pollution rate and sprouting rate have no change in proper concentration of sucrose (20-25g/L). The difference between the treatments ($P < 0.05$) of different species was not significant; under the same concentration of sucrose, between different species being significant ($P < 0.05$) and extremely significant ($P < 0.01$), different plants have different effects on sucrose. Other previous experiments also prove that high concentrations of sucrose can enhance the content of secondary metabolites in cell culture, can improve the fern spore sprouting rate and accelerate the germination speed and promote the growth of prothallium. One of the reasons is raising the culture medium osmolarity, then stimulating the secondary product formation (Gan Fanyuan et al.1997; Bao Min et al.2000; Li Tianzhen et al.2001; Rahmatullah Jan

et al.20020; Palma, J.M. et al.2023). The result shows that the sucrose concentration is not a critical factor for the determination of sprouting rate, but an indispensable nutrition. Just as what has been found by Gan Fanyuan et al. (1997) and Gao Yongchao et al. (2003), during their culture of *Taxus yunnanensis*, when the sucrose concentration was less than 20 ~ 30g /L, the growing speed of the cultured cells declined rapidly.

Recently, many studies have reported Traditional Medicinal Herbs as rich sources of novel anticancer drugs (Cai Y Z et al.2004;Wu Yang Huang et al.2009; Li Jinghong et al.2022; Li Jinghong et al.2024), and the plant tissue culture are important to multiply and conserve the critical genotypes of medicinal plants (Abdin,M. Z. et al.2007; Khan,M.Y.et al.2009; Chen, J. et al.2022;Sivanandhan, G.et al.2023).It is used as an advanced biotechnological system tool for the production of high quality plant-based medicines and the modern pharmaceutical industry also requires a large quantity of authentic plants for manufacture of drugs(Hrudayanath Thatoi et al.2011;Li Jinghong et al.2018).In tissue culture production applications, it is also necessary to consider the role of medium components, plant growth regulator, pH value, illumination, CO_2 concentration and other factors (Yang Chunyan.et al.2006; Qun Jiangbing et al.2009; Nezami Alanagh, M. et al.2020; Zhao, X. 2021; Zhang, Y. et al.2021; Ahmad, N. et al.2022;Wang, X.et al.2023), different species or varieties in the tissue culture at different stages of culture conditions are not the same. Therefore, we first study the effect of the same factor on the plant explants through the single factor experiment. On this basis, we will carry out the multi-factor experiment in the future, and find out the main effect of several factors by rationally allocating these influencing factors and the interaction effect, so as to screen out the optimal combination of culture conditions for plant explants, which can ensure the good growth and development of plant tissue culture seedlings, and finally provide the theoretical basis for the production of high quality seedling of medicinal plants.

Statements & Declarations

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Competing interests

This original research paper was completed through a series of experimental studies.

The authors declare that they have no competing interests.

Authors’ contributions

LI Jing-Hong conceived and designed the experiments, performed the experimental work, and drafted the manuscript; XIAO Di assisted with the experimental work, data analysis, and manuscript writing; Chen Gong-xuan contributed to manuscript revision. All authors have read and approved the final version of the manuscript.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

Not applicable.

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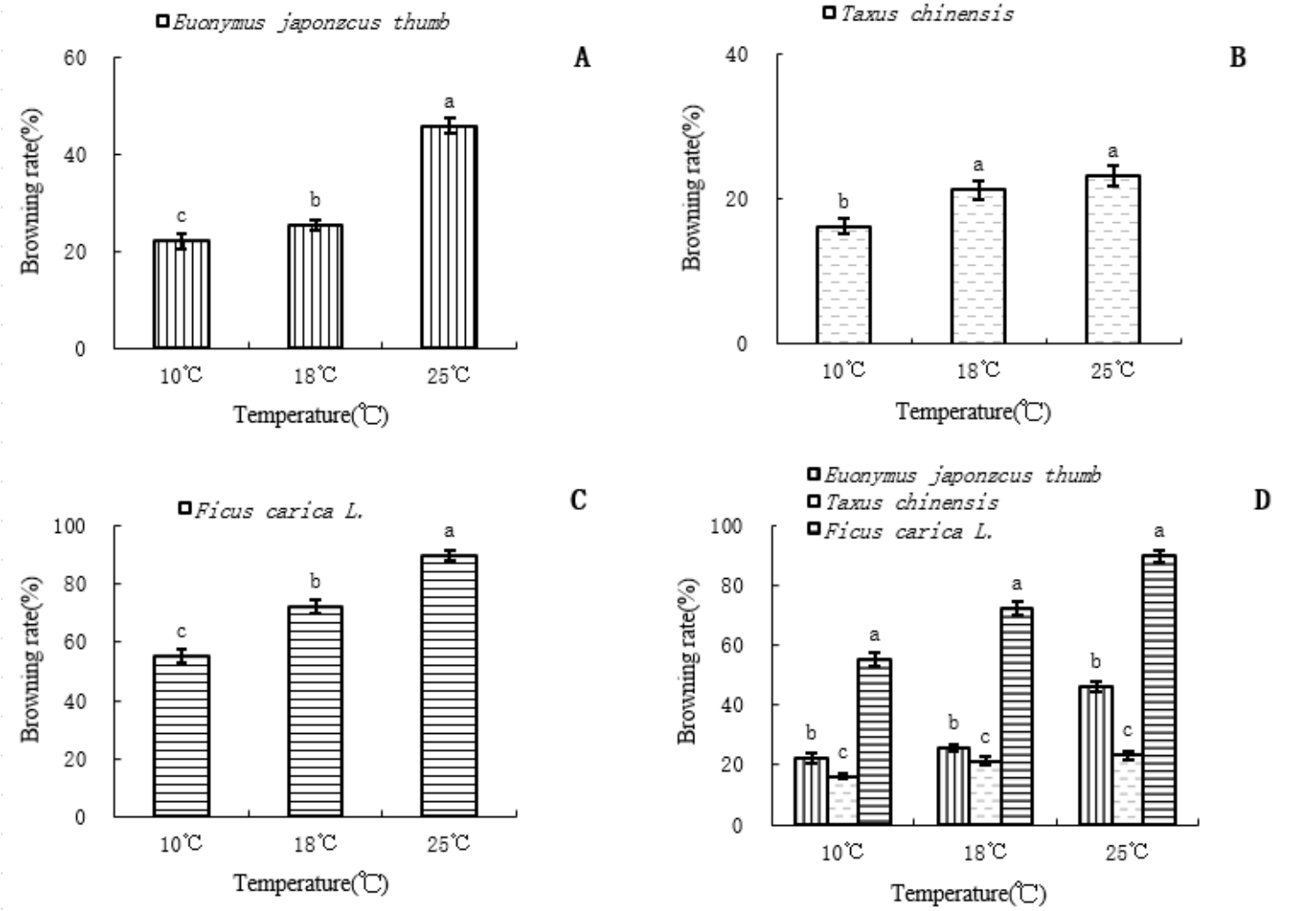
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Figures



Notes: In figure A, B, and C, different letters mean that there is significant difference in temperature treatment ($P < 0.05$);In figure D, at the same temperature, different letters indicate a significant level between species ($P < 0.05$), according to Duncan's multiple range test. The same as in the figures below.

Figure 1

The Influence of Different Temperatures on the Browning of Explant

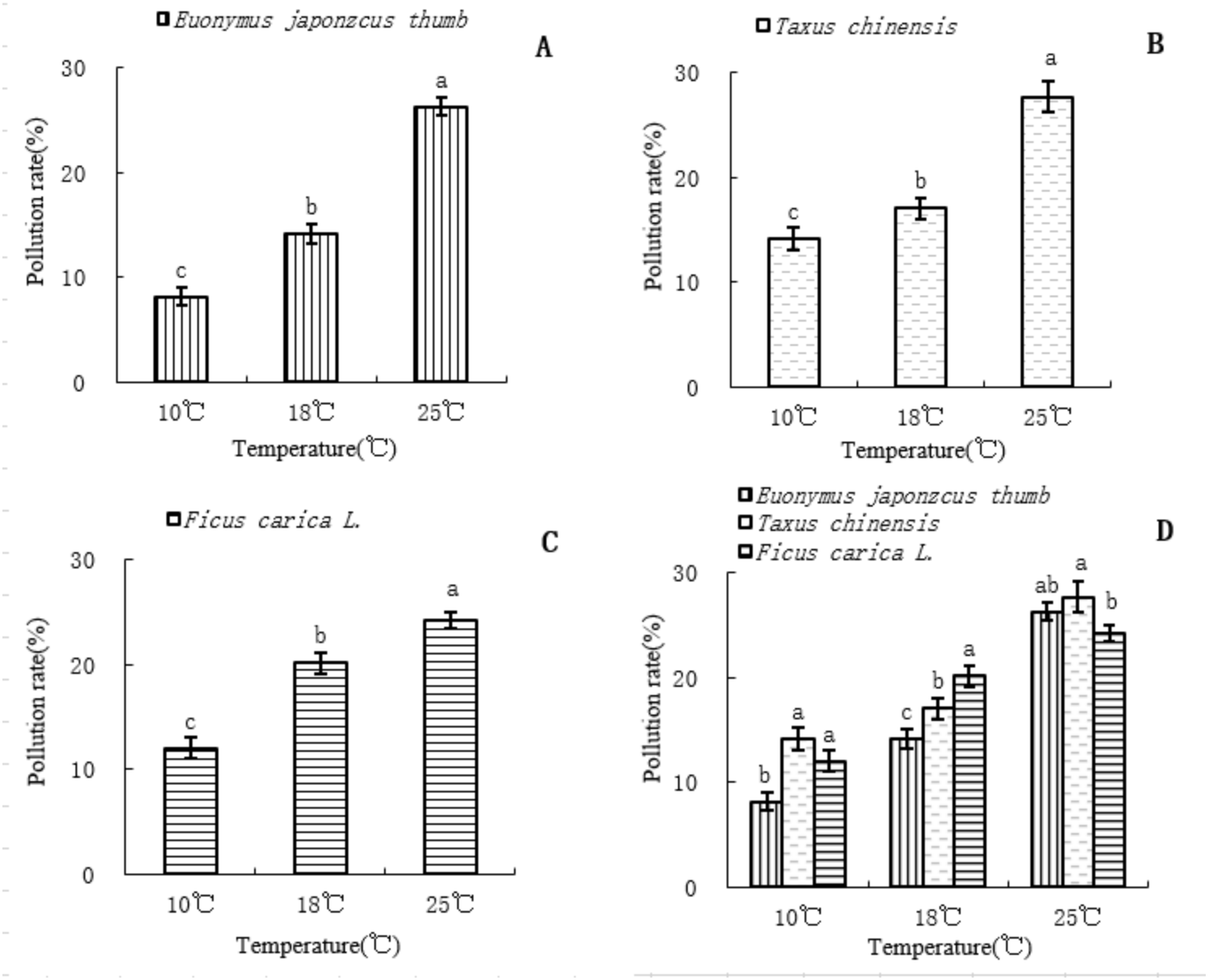


Figure 2

The Influence of Different Temperature on Pollution of Explants

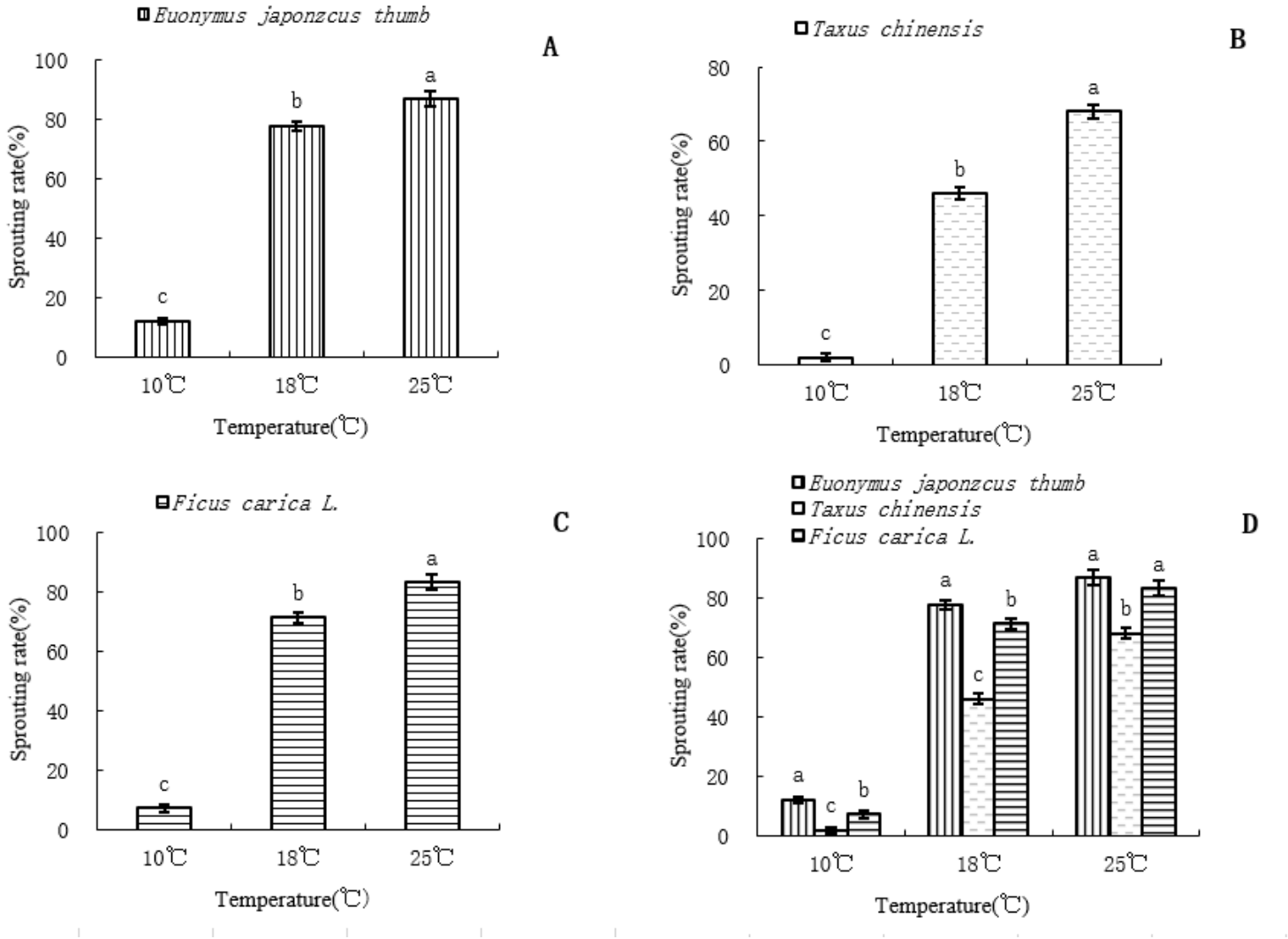
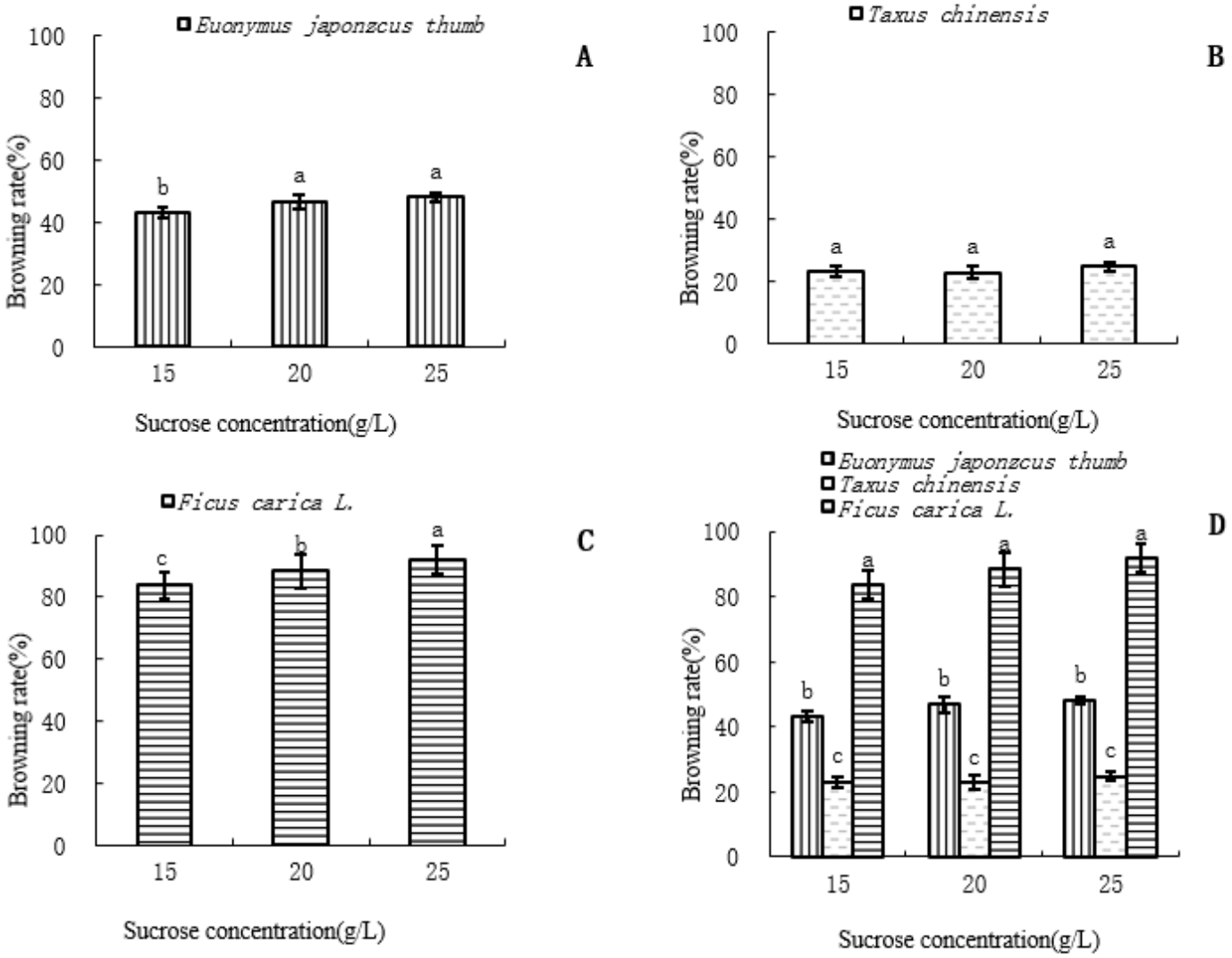


Figure 3

The Influence of Different Temperature on Sprouting of Explants



Notes: In figure A, B, and C, different letters mean that there is significant difference in sucrose concentration treatment ($P < 0.05$);In figure D, at the same sucrose concentration, different letters indicate a significant level between species ($P < 0.05$), according to Duncan's multiple range test. The same as in the figures below.

Figure 4

The Influence of Sucrose Concentration on Browning of Explants

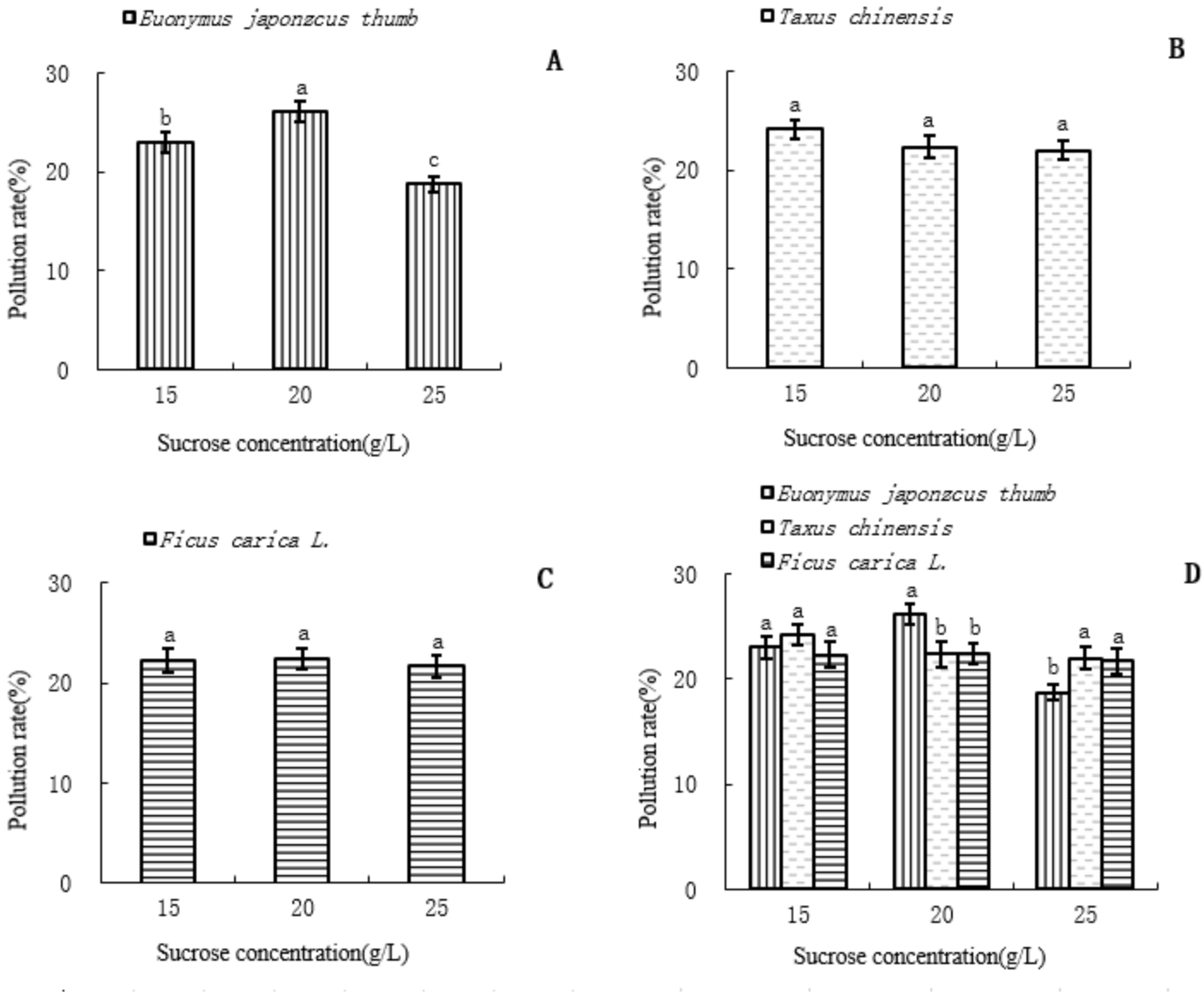


Figure 5

The Influence of Sucrose Concentration on Pollution Rate of Explants

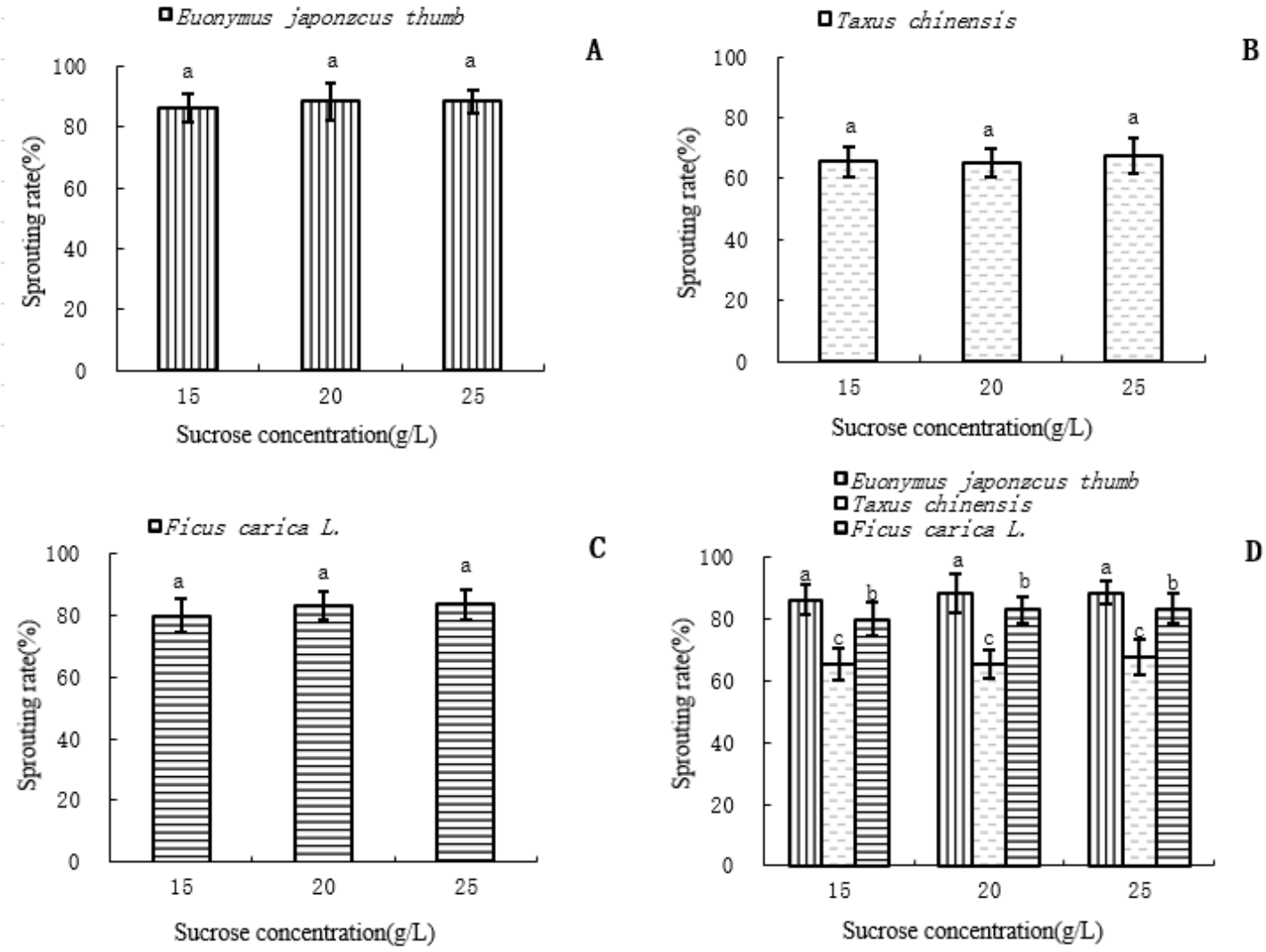


Figure 6

The Influence of Sucrose Concentration on Sprouting Rate of Explants