

Somatic embryogenesis processes and changes in endogenous hormone content of *Cinnamomum camphora* L.

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Abstract

Immature zygotic embryos of the camphor tree were used as explants, under dark conditions, the somatic embryos induction rate (68.75%) was the highest when the immature zygotic embryos were 6 mm in size. And using sucrose as a carbon source was more conducive to the induction of somatic embryos. The highest cotyledon embryos proliferation rate (36.67%) appeared in the combination of 1.2 mg/L 6-BA + 0.2 mg/L IBA + 8.0 mg/L GA₃, and the highest cotyledon embryos germination rate (20%) appeared at 2.4 mg/L 6-BA + 0.4 mg/L IBA + 4.0 mg/L GA₃. Changes of endogenous hormone content and ratio of various types of embryogenic cultures were clearly diverse: IAA and ABA were always at higher levels, and the highest values of GA₃ (0.31 ng·g⁻¹) and ZT (0.09 ng·g⁻¹) appeared in SEG explant, and ZT was only detected in SEG explant, it is speculated that appropriately increasing the content of endogenous ZT or the concentration of exogenous cytokinin may help to induce germination of somatic embryos. The highest values of ABA/IAA (8.46) occurred in SEG explant, followed by SE explant (2.83), which was initially thought to be related to the development and germination of somatic embryos. The highest values of ABA/GA₃(180.00) were found in SE explant, followed by YC explant (69.50), which indicated that a higher ratio was beneficial to the normal development of somatic embryos.

Introduction

Cinnamomum camphora also known as the camphor tree, is a large evergreen tree in the camphor family, a representative tree species of subtropical evergreen broad-leaved forests, mainly distributed in China south of the Yangtze River and southwest China (Zhou and Yan 2005). Its well-developed crown, dense branches, and leaves, shade and shelter from the sun, is an excellent urban greening tree, shade tree (Tian et al.2004; Zhang et al. 2017; Xu et al.2022). In recent years, with the continuous development of urban garden construction, camphor tree has been more and more widely used in urban landscaping, and many gardeners have tried to introduce this species to north China (Wang 2010; Hu et al. 2015). However, with the warm nature of camphor tree, it can suffer from frost damage in northern winter conditions, even causing tree death, resulting in huge economic losses. Therefore, how to prevent and control the damage caused by low temperatures to camphor tree has become the key to the application and promotion of this tree species in the north. The problem can be solved by strengthening the cultivation management and breeding new varieties, but the long growth cycle and complex genetic background of woody plants make the process of conventional breeding to improve tree species slow and inefficient, while molecular breeding (MB) has the advantages of the purposeful and short breeding cycle, which can improve the plant tolerance in a short time.

Molecular breeding has become an integral part of breeding work due to its advantages of improving traits and breaking through species isolation (Parry et al.2012; Bai et al. 2018; Li et al.2021). In some woody plants such as *Pinus taeda* L. (Tang et al.2005), *Eucalyptus globulus* (Matsunaga et al.2012), and *Betula platyphylla* (Lv et al.2019), the breeding goal of improving stress resistance has been initially achieved through transgenic techniques. Lv(2019)used birch leaves as explants, and through agrobacterium transformation, the transgenic lines overexpressed *BpERF13*, showing a normal

phenotype and significantly enhanced tolerance to cold stress. Therefore, the use of molecular breeding techniques to achieve the stress resistance of the woody plant camphor tree is feasible, however, a stable and efficient plant regeneration system is the prerequisite for the success of molecular breeding.

Currently, the regeneration protocols available in studies on the regeneration of camphor tree plants include organogenesis and somatic embryogenesis with cotyledon embryos as explants (Shi et al.2010; Du et al. 2014; Du et al.2015; He et al. 2015). The regeneration method of somatic embryogenesis has the advantages of genetic stability and a high regeneration rate compared with organogenesis, and is an important method for the genetic improvement of forest trees, thus it has been widely used. Du (2006) and Dai (2019) used immature zygotic embryos of camphor tree as explants to first induce embryogenic callus and then used embryogenic callus as explants to induce somatic embryos to achieve plant regeneration through the somatic embryogenesis pathway. In addition, the hypocotyl of the zygotic embryos can also be directly induced to produce plants (Jing et al. 2019).

During embryonic development, endogenous hormones play a key role. Therefore, the relationship between changes in endogenous hormone content and somatic embryogenesis in vitro has also been the subject of many studies (Farias-Soares et al. 2014). Several studies have shown that auxin (IAA) is essential for the establishment and development of somatic embryo polarity, and the enrichment of IAA facilitates somatic embryogenesis (Zhou et al. 2017; Liu et al. 2019). Like auxin, cytokinins (CKs) are also the key to somatic embryo induction. In the study of somatic embryogenesis in *Begonia*, it was found that high levels of CKs and the ratio of CKs/ABA could increase the number of somatic embryos by 1.57 times compared with the control (Khai et al. et al.2021). Absciscic acid (ABA) is mainly associated with the synthesis of storage material at the maturation stage (Su et al. 2013). Liang (2022) found that high levels of ABA in Korean pine were beneficial to facilitate early somatic embryo differentiation. Kępczyńska (2021) found that GAs are involved in the acquisition of embryogenic competence in *Medicago truncatula* leaf somatic cells, however, Jiménez (2005) found no differences in GAs levels in the study of cultures with different embryonic developmental characteristics of carrots. It can be seen that there are genotypic differences in the effects of gibberellins (GAs) on somatic embryogenesis.

At present, although the plant regeneration system of *Cinnamomum camphora* has been researched and established, the changes of endogenous hormones content during somatic embryogenesis of *Cinnamomum camphora* have not yet been reported based on the induction and proliferation of cotyledon embryos. In this paper, *Cinnamomum camphora* was taken as the research object, and the zygotic embryos in immature fruits were selected as explants to induce cotyledon embryos, and induce proliferation and germination of cotyledon embryos. In addition, the endogenous hormone content of explants at different stages during somatic embryogenesis was measured, which can not only provide a reference basis for the developmental regulation of *Cinnamomum camphora* embryos, but provide new clues for the mechanism of somatic embryogenesis in *Cinnamomum camphora*.

Materials And Methods

Medium and culture conditions

In this study, MS was used as the basic medium, supplemented with 30 g/L sucrose, 8.0 g/L agar, 0.2 mg/L IBA, and 1.2 mg/L 6-BA, and this medium was recorded as T medium. The medium was sterilized at 121°C for 20 min after pH adjustment to 6.0. T medium was used for the culture of immature zygotic embryos, the proliferation of cotyledon embryos, and the proliferation of somatic embryo germination. Hormone-free MS medium containing 30 g/L sucrose and 8.0 g/L agar was used as the rooting medium for rootless embryo seedlings. Unless otherwise specified, the explants were cultured at $24 \pm 2^\circ\text{C}$ under a 16 h photoperiod with $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiations supplied by white fluorescent lights.

Induction and proliferation of cotyledon embryos in *C. camphora*

In mid-July, immature fruits of about 1 cm in diameter were harvested from adult camphor trees without pests and diseases on the campus of Nanyang Normal University as the research material. After rinsing the camphor trees immature fruits in running water for 1 h, they were placed in a solution of 75% alcohol by volume for 30 s, then in 0.1% mercuric chloride solution for 8 min, and rinsed 3–5 times with sterile water in the ultra-clean bench. Afterward, the fruits were placed on sterile filter paper with tweezers, cut with a scalpel perpendicular to the fruit pedicle and 2/3 away from the fruit pedicle, and the complete cotyledon embryo peeled out with a dissecting needle at the far end of the fruit pedicle. Set different zygotic embryo sizes (about 2 mm, 4 mm, and 6 mm), different culture conditions, and 30 g/L of different carbon sources (sucrose, glucose, sorbitol, mannitol) for cotyledon embryo induction experiments. 4 dishes in each treatment, 16 per dish, and the induction of cotyledon embryos were recorded after 3 weeks.

Select the creamy white, fleshy, and thick single cotyledon embryo, and chopped perpendicular to the single cotyledon embryo vertically and horizontally with two knives. The fragments were gathered into 0.5 cm clumps (about 5–7 fragments) and inoculated in T medium, cultured in the dark. After the cotyledon embryos were proliferated every 2 weeks using the same method. Somatic embryo induction rate = (total number of explants induced with somatic embryos / total number of inoculated explants) $\times$ 100%; Somatic embryo induction coefficient = (total number of somatic embryos induced / total number of inoculated explants) $\times$ 100%; Embryo bud induction rate = (total number of explants induced with buds / total number of inoculated explants) $\times$ 100%.

Germination of cotyledon embryos in *C. camphora*

The same operation was used for the above-mentioned cotyledon embryos' clonal lines with better proliferation effect, with different 6-BA concentrations (1.2 mg/L, 2.4 mg/L), IBA concentrations (0.2 mg/L, 0.4 mg/L) and GA3 concentration (0 mg/L, 2 mg/L, 4 mg/L, 8 mg/L) for germination induction of cotyledon embryo, and cultured in the light, 2 dishes in each treatment, 15 per dish, continuous culture for 4–5 weeks, the budding induction of somatic embryos were recorded. were cultured continuously for 4–5

weeks and the induction of somatic embryo germination was recorded. Somatic embryo proliferation rate = (total number of explants proliferating out of somatic embryos/total number of inoculated explants) × 100%; somatic embryo germination induction rate = (total number of explants inducing out of somatic embryo germination /total number of inoculated explants) ×100%.

Select robust somatic embryo germination and transfer them to T medium in time and cultured in light for 2 weeks for the proliferation of embryo buds.

Determination of endogenous hormones ZT, GA3, IAA, and ABA

Weigh 1g fresh samples of *C. camphora* (somatic embryo, yellow callus, and somatic embryo germination) and grind them to the dry powder in liquid nitrogen, add isopropanol-water-hydrochloric acid mixed extract and 8μL 1μg/mL internal standard solution, shake at low temperature for 30min; Then add dichloromethane, shake at low temperature for 30min. After centrifugation (13000 r/min) for 5 min at 4°C, the lower organic phase was removed. The organic phase was blown dry with nitrogen in the dark, reconstituted with methanol (0.1% formic acid); centrifuged at 4°C (13000 r/min) for 10 min, and the supernatant was taken through a 0.22 μm filter membrane for HPLC-MS/MS detection.

HPLC conditions: Column: Poroshell 120 SB-C18 reversed-phase column (2.1×150, 2.7μm); column temperature: 30°C; mobile phase: A: B=(methanol/0.1% formic acid): (water/0.1% formic acid); elution gradient: 0–1 min, A = 20%; 1–9 min, A increasing to 80%; 9–10 min, A = 80%; 10-10.1 min, A decreasing to 20%; 10.1–15 min, A = 20%; injection volume: 2 μL. MS-MS conditions: Electrospray ionization source ESI atomization temperature: 400°C, gas curtain gas: 15 psi, spray voltage: 4500 V, atomizing gas pressure: 65 psi, auxiliary gas pressure: 70 psi, monitoring mode: MRM (Multi-Reaction Monitoring Mode). In the Q-Trap6500, each ion pair is scanned according to the optimized declustering potential (DP) and collision energy (CE). The detection of plant endogenous hormones was completed by Convinced Technology Ltd.

Statistical analysis

The dates collected from the experiments were calculated and analyzed in SPSS Statistics 22.0 and Origin 2019 software with Duncan's multiple range test at the level of significance P = 0.05. Percentage data were transformed *via* arcsine before analysis.

Results

Effects of Different sizes, culture conditions, and carbon sources on induction of cotyledon embryos

Zygotic embryos (Fig. 1A) from immature fruits of camphor trees were cultured in T medium for 3 weeks, and cotyledon embryos were induced under both dark and light conditions. The zygotic embryos cultured

in dark conditions were enlarged and thickened, and young creamy white cotyledon embryos can be observed at the articulation of the double cotyledons, which appear in clusters and form a spiral around the longitudinal axis of aggregation (Fig. 1B). The zygotic embryos in light culture swollen and turn green, inducing white clustered cotyledon embryos (Fig. 1C). The somatic embryos under dark conditions were fleshy and thick, while those under light conditions are white and flaky. Direct germination of zygotic embryos (Fig. 1D), induction of somatic embryos and shoots from zygotic embryos (Fig. 1E), and seed-like germination from zygotic embryos (Fig. 1F) were also observed under light culture conditions.

Under different culture conditions, zygotic embryos of different sizes were induced differently (Table 1). Under light conditions, the somatic embryo induction rate of 4 mm zygotic embryos was the highest at 53.13%, and the somatic embryo induction coefficient was also the highest, reaching 3.71. Under dark conditions, the induction rate of somatic embryos with a size of 6 mm was the highest, which was 68.75%, and the somatic embryos grew better under dark culture than under light. Therefore, when using immature zygotic embryos as explants to induce cotyledon embryos, a size of 6 mm is appropriate. It is easier to obtain embryo buds by selecting 2 mm zygotic embryos for light culture. Under dark conditions, zygotic embryos of all sizes are more likely to induce somatic embryos, and easily induce callus, but not easily induce embryo buds.

Zygotic embryos of 6 mm in size were selected and placed in T medium to study the effects of different carbon sources on the induction of cotyledon embryos (Table 2). The results showed that sucrose was more favorable to the induction of somatic embryos, with a 68.75% induction rate of somatic embryos, and it also easily produced callus; glucose and mannitol had similar induction rates of somatic embryos, but sorbitol was not effective in the induction of somatic embryos, the somatic embryo induction coefficient was similar in sucrose and glucose. Therefore, when using immature zygotic embryos as explants to induce cotyledon embryos, the best choice is sucrose as the carbon source.

In the exploration of the effects of different sizes, culture conditions, and carbon sources on the cultivation of immature zygotic embryos of camphor trees, it can be seen that the somatic embryos induced under light and dark culture conditions are different in shape, with the somatic embryos under dark conditions being tender and thick. Dark culture as a condition, zygotic embryos with a size of 6 mm, and sucrose as a carbon source were suitable for somatic embryo induction. Dark conditions were used as the culture conditions in the subsequent culture of cotyledon embryos.

A. Zygotic embryo, bar: 2 mm; B. Cotyledon embryos induced from zygotic embryos under darkness, bar: 2 mm; C. Cotyledon embryos induced from zygotic embryos under light, bar: 2mm; D. Zygotic embryo germination under light, bar: 2 mm; E. Malformed germination of the zygotic embryo under light, bar: 2 mm; F. Seed-like germination from the zygotic embryo, bar: 5 mm.

Table 1

Effects of different sizes and culture conditions on cotyledon embryos induction of *C. camphora*

Culture Condition	Zygotic embryos size	Somatic embryos induction rate	Somatic embryo induction coefficient	Embryo buds induction rate	Embryonic callus induction rate
		(%)		(%)	(%)
Light culture	2 mm	37.50 ± 5.12b	2.58 ± 0.12c	12.50 ± 2.12a	4.69 ± 1.57b
	4 mm	53.13 ± 5.77a	3.71 ± 0.35a	6.25 ± 1.67b	7.81 ± 1.66a
	6 mm	32.81 ± 5.01b	3.10 ± 0.12b	3.13 ± 0.35c	7.81 ± 1.47a
Dark culture	2 mm	45.94 ± 4.64b	3.13 ± 0.22a	1.56 ± 0.12a	21.88 ± 1.55b
	4 mm	45.63 ± 4.12b	3.17 ± 0.15a	0.00 ± 0.00b	23.44 ± 2.12b
	6 mm	68.75 ± 7.64a	2.92 ± 0.22a	1.56 ± 0.13a	28.13 ± 2.01a

Different lowercase letters in the same column indicate $P < 0.05$

Table 2

Effects of different carbon sources on cotyledon embryos induction of *C. camphora*

Carbon Sources	Somatic embryos induction rate	Somatic embryo induction coefficient	Embryo buds induction rate	Embryonic callus induction rate
	(%)		(%)	(%)
Sucrose	68.75 ± 9.01a	2.92 ± 0.22a	1.56 ± 0.11c	28.13 ± 3.15a
Glucose	52.50 ± 8.55b	3.2 ± 0.35a	6.25 ± 0.57a	12.15 ± 1.49b
Sorbitol	15.63 ± 3.36c	1.8 ± 0.10b	3.25 ± 0.22b	0.00 ± 0.00d
Mannitol	46.88 ± 6.77b	2.53 ± 0.12a	3.38 ± 0.42b	6.25 ± 1.28c

Different lowercase letters in the same column indicate $P < 0.05$

Proliferation and germination induction of cotyledon embryos of *C. camphora*

The camphor tree cluster embryos obtained under dark conditions were selected, and the single-piece embryos were obtained by separating them one by one at the longitudinal axis. The single piece of embryos was cut into 2–3 mm pieces as the material for somatic embryo proliferation. The somatic embryo pieces were aggregated into 5 mm clumps and inoculated in T medium for dark culture (Fig. 2A), about 2 weeks, densely arranged and clustered granular embryos (Fig. 2B) and clustered cotyledonary somatic embryos (SE explants) were observed at the wound of the fragment (Fig. 2C), and the proliferation rate of somatic embryos about 46.4%. After that, using the same method, subculture every 2 weeks to complete the proliferation of cotyledon embryos. Through the above method, timely subculture (timely subculture: once every 10–15 days) and proliferation of somatic embryos from the same source

can establish cotyledon embryos' clonal lines. Somatic embryo germination (SEG explants) was observed on chopped somatic embryos after delayed subculture (delayed subculture: once every 20–30 days) in the process of proliferating somatic embryos (Fig. 2D). At the same time, the callus of the fragments, adventitious buds growing on the surface (Fig. 2E) and induction of yellow embryogenic callus (YC explants) were also observed (Fig. 2F).

There were differences in the germination of somatic embryos under different combinations of PGRs (Table 3). The optimal combination of PGRs for the germination of cotyledon embryos was 2.4 mg/L 6-BA + 0.4 mg/L IBA + 4.0 mg/L GA₃, with a germination rate of 20%. Based on 6-BA and IBA application, there was a tendency for the germination rate of somatic embryos to increase and then decrease with increasing GA₃ content. In addition, the proliferation rate of somatic embryos showed a continuous increase with increasing GA₃ content, reaching the highest rate of 36.67% in the combination of 1.2 mg/L 6-BA + 0.2 mg/L IBA + 8.0 mg/L GA₃. Therefore, to induce somatic embryo germination by cotyledon embryos as explants under light culture, the addition of 4.0 mg/L GA₃ was preferred based on a 6/1 ratio of 6-BA to IBA application; the addition of 8.0 mg/L GA₃ was selected to proliferate somatic embryos more easily. Callus was easily induced under all PGR combinations.

A. Chopped-somatic embryos, bar:2 mm; B. Granular embryos induced from the chopped-somatic embryo, bar: 2 mm; C. Cotyledon embryos propagation, bar: 2 mm; D. Chopped-somatic embryo germination, bar: 5 mm; E. Malformed germination of the callus-somatic embryo, bar: 5 mm; F. Yellow embryogenetic callus induced from the chopped-somatic embryo, bar: 2 mm.

Table 3
Effects of different PGRs combinations on the germination of somatic embryos

PGRs concentration			Somatic embryo germination induction rate	Somatic embryos proliferation rate	Callus induction rate
6-BA	IBA	GA ₃			
(mg/L)	(mg/L)	(mg/L)	(%)	(%)	(%)
1.2	0.2	0.0	3.33 ± 0.12d	16.67 ± 2.24d	50.00 ± 9.36c
1.2	0.2	2.0	0.00 ± 0.00e	20.00 ± 2.28cd	46.67 ± 8.49cd
1.2	0.2	4.0	10.00 ± 2.21b	23.33 ± 3.21c	30.00 ± 5.77d
1.2	0.2	8.0	10.00 ± 1.79b	36.67 ± 5.49a	46.67 ± 7.35cd
2.4	0.4	0.0	3.33 ± 0.12d	13.33 ± 2.01e	63.33 ± 8.00b
2.4	0.4	2.0	6.67 ± 1.49c	20.00 ± 3.00cd	73.33 ± 11.55a
2.4	0.4	4.0	20.00 ± 3.28a	23.33 ± 3.17c	70.00 ± 11.36ab
2.4	0.4	8.0	10.00 ± 2.36b	30.00 ± 4.04b	50.00 ± 9.01c

Different lowercase letters in the same column indicate $P < 0.05$

Endogenous hormones and Endogenous hormones ratios from various types of embryogenic cultures of *C. camphora*

The growth and development of plants depend on the interaction between the effective hormones within the plant, which is the result of the combined action of multiple endogenous hormones. It is of practical significance to study the changing trend of the content of endogenous hormones for understanding and regulating the growth and development of somatic embryos. In this study, the somatic embryos (SE), yellow callus (YC), and somatic embryo germination (SEG) obtained under the same culture conditions were selected as materials, and the contents of endogenous hormones ZT, ABA, GA₃, and IAA were determined (Fig. 3). The results showed that indoleacetic acid (IAA) and abscisic acid (ABA) content were high in the three types of materials, gibberellin (GA₃) content was significantly reduced, zeatin (ZT) was only detected in SEG explants, and not in YC explants or SE explants, so it is speculated that endogenous cytokinin may have important significance for the formation of germination, which also matches the regularity of plant growth and development. The highest content of IAA in YC explants was 6.24 ng/g, and the lowest was in SEG explants. It may be due to the division and differentiation activities of the

callus, which led to the most significant effect of IAA in the callus with vigorous growth and metabolism, and the effect of auxin in SE explants and SEG explants is weakened. The four endogenous hormones showed the highest ABA content in the three types of materials, with the highest ABA content in SE explants being 14.4 ng/g and only 8.27 ng/g in YC explants. Indicating that ABA plays a key role in embryo morphogenesis and maturation, which is similar to the results of Liang. (Farias-Soares et al. 2014; Liang et al.2022). The highest content of GA3 in SEG explants was 0.311 ng/g, and the least in SE explants is 0.084 ng/g. It is speculated that GA3 has a positive effect on the release of dormancy and the germination of somatic embryos (Cui et al.2020). A small amount of ZT was detected in SEG explants, containing only 0.089 ng/g. Thus, when promoting the germination of somatic embryos, one can choose to properly increase the content of endogenous ZT or increase the concentration of exogenous cytokinin for the germination of somatic embryos.

The ratio between the hormones showed (Fig. 4) that the ratio of ABA to IAA reached the highest value at the SEG explants, which was 3–6 times higher than that of SE explants and YC explants, followed by SE explants, with the lowest ratio at the YC explants. It is preliminarily believed that the increase in the mass ratio of ABA to IAA may be beneficial to the development and germination of somatic embryos of camphor trees. The ratio of ABA/GA3 was the highest at the SE explants, while the ratio of ABA/GA3 decreased significantly in the YC explants and SEG explants. This indicates that a higher mass ratio of growth-inhibiting substances to growth-promoting substances is beneficial for the normal development of camphor tree somatic embryos, which is similar to the results of tea tree studies (Guo et al.2018). At the same time, it also shows that the demand for various types of endogenous hormones varies greatly at different stages.

Discussion

As an expression of plant cell totipotency, somatic embryogenesis is the process by which new individuals are formed from plant somatic cells under in vitro conditions through a developmental pathway similar to that of the syncytial embryo (Ravishankar & McComb, 2002). Compared to organogenesis, somatic embryogenesis has the advantages of large quantity, fast speed, and genetic stability (Gupta et al, 1993; Xu et al, 2020), so it has become the main method of plant regeneration in recent years. Camphor trees immature zygotic embryos have also previously been shown to be easier explants for the somatic embryo induction pathway (Du et al, 2014; Jing et al, 2019).

There are many factors affecting the induction of somatic embryos, such as the time of explant collection, genotype, type and concentration of growth-regulating substances added to the medium, etc. Most scholars have done many studies on induction factors, but there can still be differences of opinion, which may be related to many factors such as the genotype and physiological age of the explants. In the study on the effect of induction of somatic embryos in camphor trees, Yang (2020) found that the best sampling period was July when studying the effect of different sampling periods on germination; Chen (2014) found that young camphor trees embryos collected at different times had a significant effect on somatic embryogenesis. It can be seen that the collection of young camphor tree embryos in July is very

important for somatic embryo induction. In this study, the immature zygotic embryos of *Cinnamomum camphora* collected in July were used as explants to conduct somatic embryo induction and proliferation research. During this process, it was found that the somatic embryos with a size of 4 mm had the best somatic embryo induction under dark conditions, the somatic embryos appeared in clusters and the cotyledons were plump, the results of the study were similar to the previous ones. And using the cotyledon embryos as explants to induce the germination of somatic embryos, it was found that the optimal combination of PGRs for germination of cotyledon embryos was 2.4 mg/L 6-BA + 0.4 mg/L IBA + 4.0 mg/L GA3.

In addition, carbohydrates are an important carbon source, which not only regulates the osmotic pressure in the cells, but provides the energy required for the growth and metabolism of the culture, and plays a very key role in the process of plant tissue culture (Bartos et al, 2018). Commonly used carbon sources include sucrose, glucose, maltose, sorbitol, mannitol, etc. Previous studies have shown that treatment with high concentrations of sucrose can influence the production of effects similar to those of ABA, regulating the formation of somatic embryos, and increasing mannitol and polyethylene glycol can also have the same effect (Linossier et al, 1997; Jia et al, 2013). 30 g/L sucrose is a suitable concentration for most forest trees cultured in vitro (Kim et al, 2011; Hazubska et al, 2016; Song et al, 2018). In this study, the highest induction rate of somatic embryos was found when sucrose was used as the carbon source, which is the same as the previous study. Therefore, sucrose is the best choice for the subsequent induction of cotyledon embryos using immature zygotic embryos as the explants.

The reasons for obtaining different experimental results under the same culture conditions may depend on the influence of endogenous hormones in the material. The growth and development of plants are regulated by both external and internal factors. Studies on various plants have shown that organ differentiation is mainly determined by the balance of endogenous cytokinin and auxin in explants (Pescador et al, 2012). Endogenous hormones regulate gene activity, thereby affecting a series of metabolic processes, ultimately affecting the induction, maintenance, and expression of embryogenic potential in plant cells. In this study, the content of endogenous hormones ZT, GA3, IAA, and ABA was determined in three materials (yellow callus, somatic embryos, and somatic embryo germination) obtained in somatic embryo proliferation culture. It was found that ABA plays a key role in the morphological establishment and maturation stages of somatic embryos, and it was hypothesized that GA3 has a positive effect on the release of dormancy and the germination of somatic embryos. Only a small amount of cytokinin was detected in the somatic embryo germination, further confirming that the formation of induced buds requires internal cytokinin to play a leading role, which matches the regularity of plant growth and development. Thus, when promoting the germination of somatic embryos, one can choose to properly increase the content of endogenous ZT or increase the concentration of exogenous cytokinin for the germination of somatic embryos. In this study, the influencing factors of somatic embryo induction are not fully explored, and the determination of endogenous hormone content needs to be refined, and further studies will be conducted around the existing shortcomings.

Abbreviations

MS	Murashige and Skoog medium
BA	6-benzyladenine
IBA	Indole-3-butyric acid
GA3	Gibberellic acid

Declarations

Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files).

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Author Contributions

HG: Writing—original draft, Visualization; JL: Investigation, Methodology, Data curation; YM: Investigation, Validation; ZZ: Software, Methodology; LD Writing—review & editing, Funding acquisition.

Conflict of interest

The author declares that they have no conflict of interest.

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Figures

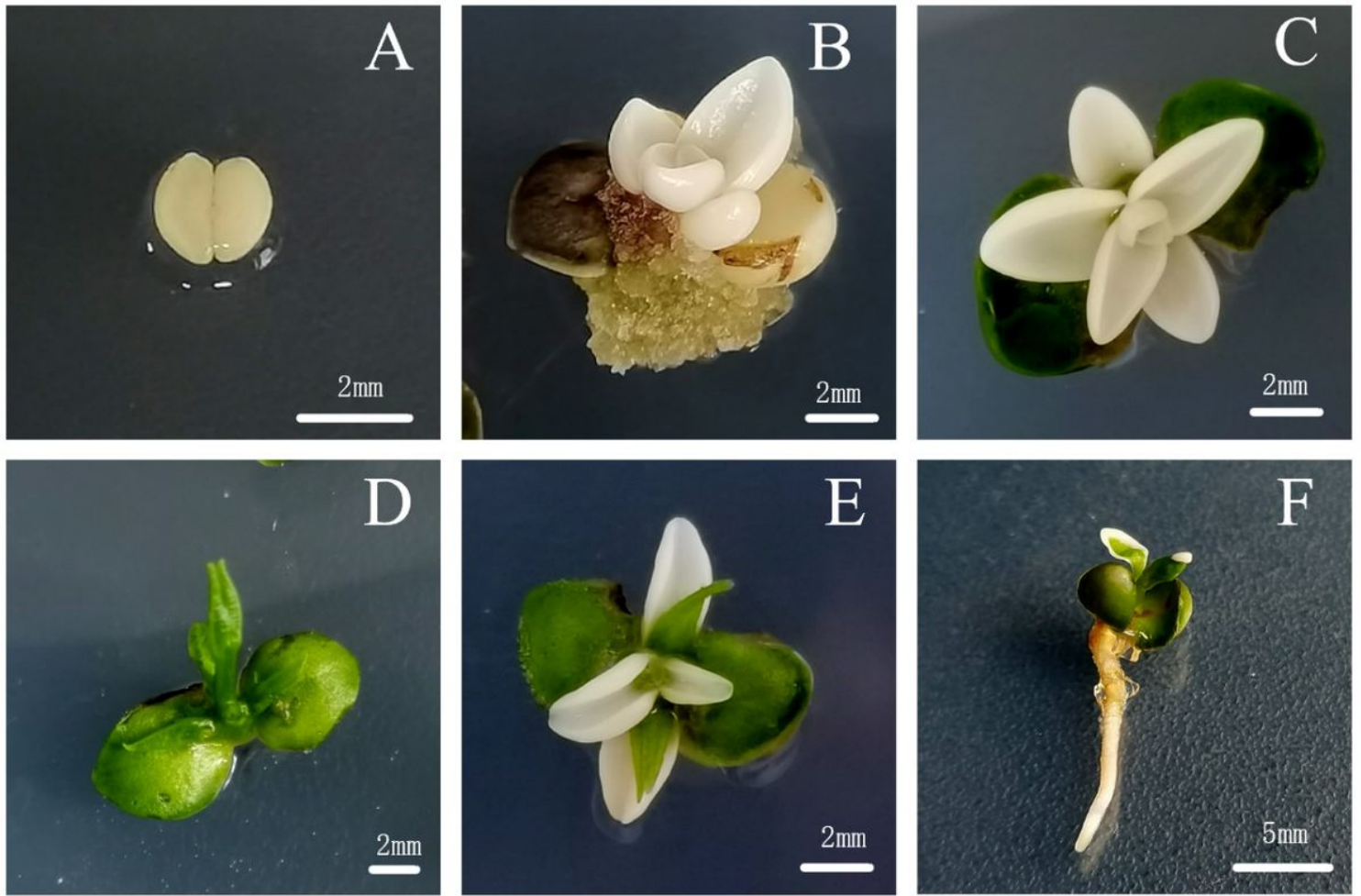


Figure 1

Cotyledon embryos induction of *C. camphora*

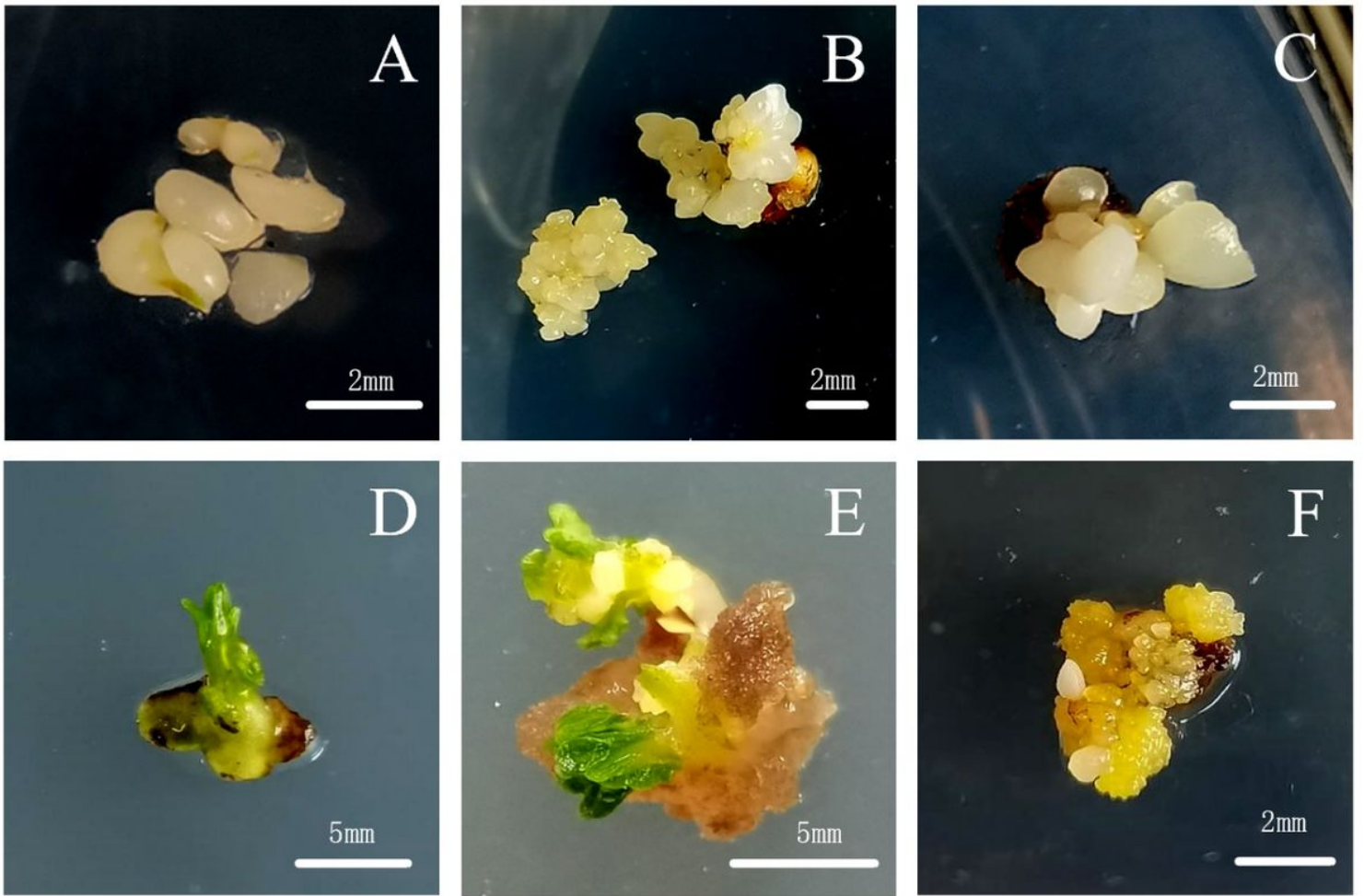


Figure 2

Cotyledon embryos propagation and germination of *C. camphora*

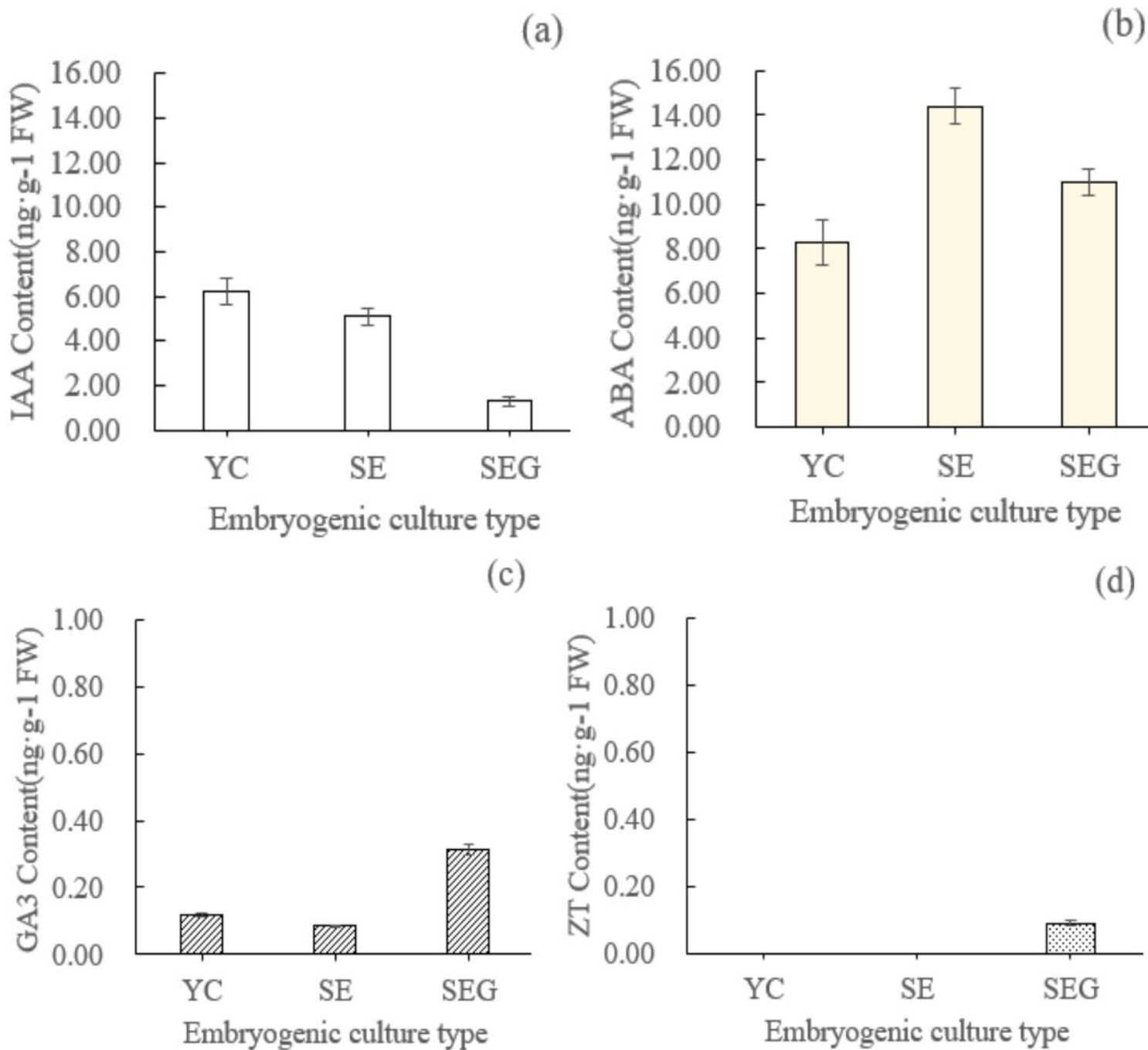


Figure 3

Determination of endogenous hormones IAA(a), ABA(b), GA3(c) and ZT(d)

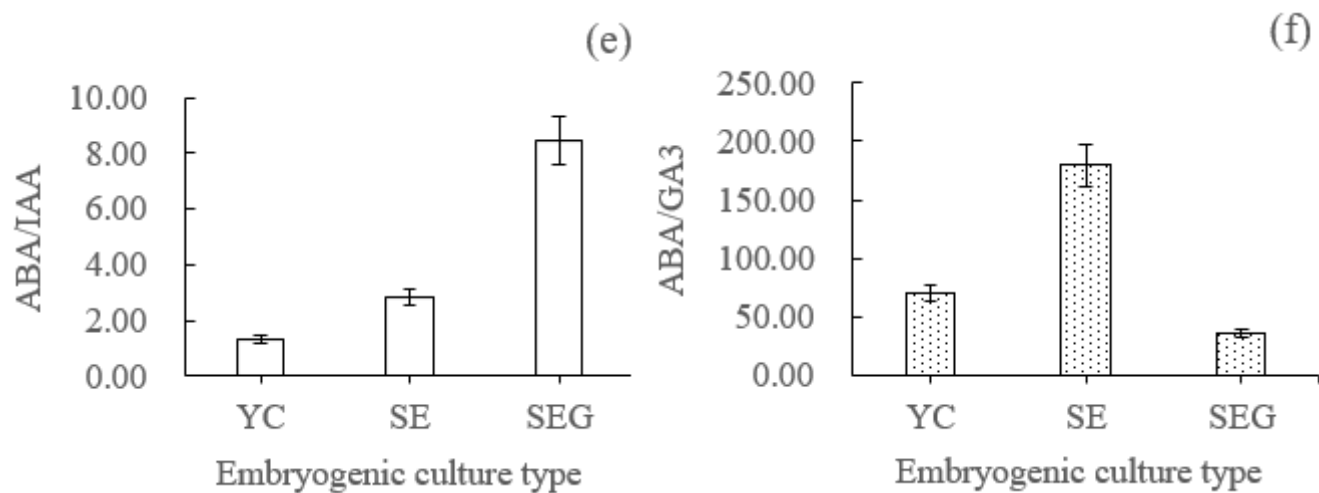


Figure 4

Changes of ABA/IAA(e) and ABA/GA3(f) during somatic embryogenesis in *C. camphora*