Tissue Culture Nursery Technology of *Curcuma alismatifolia* 'Kimono Rose'

Mingrui LIN, Zhicheng YU, Jinshui LIN, Kunxiu CAI, Zhaoyang HUANG, Xuejiao HE*

Fujian Institute of Tropical Crops, Zhangzhou 363001, China

Abstract [**Objectives**] The paper was to study the tissue culture nursery technology of *Curcuma alismatifolia* 'Kimono Rose'. [**Methods**] By sterilizing and inoculating explants derived from disparate regions of *C. alismatifolia*, we identified the most optimal explants and optimized the culture conditions for cluster buds induction and proliferation. This was achieved by incorporating MS medium with varying concentrations of 6-BA and NAA, thereby establishing a foundation for the large-scale production of *C. alismatifolia* tissue culture seedlings. [**Results**] The optimal explant for *C. alismatifolia* was identified as a lateral bud. The most effective cluster buds induction medium was determined to be MS + 6-BA 5 mg/L + NAA 0.1 mg/L + sucrose 25 g/L + agar 6.5 g/L. The optimal cluster buds proliferation medium was found to be MS + 6-BA 3 mg/L + NAA 0.1 mg/L + sucrose 25 g/L + agar 6.5 g/L. [**Conclusions**] The findings of this study can provide a foundation for the enhancement of the industrialized breeding system of tissue culture propagation of *C. alismatifolia*.

Key words *Curcuma alismatifolia*; Explants; Cluster buds induction; Proliferation

1 Introduction

Curcuma alismatifolia is a flowering bulb belonging to the genus *Curcuma* in the family Zingiberaceae. It is native to Southeast Asia and is characterized by sterile bracts that serve as its primary ornamental components. Its morphology bears resemblance to that of tulips, which have been designated as the "tropical tulips". The distinctive floral morphology, vibrant hues, extended blooming period, and other characteristics of *C. alismatifolia* indicate promising market potential. The propagation of *C. alismatifolia* is currently predominantly achieved through seedballs, a method that is associated with several disadvantages, including low efficiency, a high risk of diseases and pests, and susceptibility to environmental influences. The use of tissue culture technology for seedling production allows for the creation of high-quality seedlings in a relatively short period of time, ensuring both quality and quantity. To date, some tissue culture studies have also been conducted on *C. alismatifolia* and *Curcuma* plants. For example, Mou Xiaoling *et al.*[1] successfully induced the formation of cluster buds on a medium containing MS + NAA at a concentration of 0.02 mg/L by using small buds derived from *C. alismatifolia* bulbs as explants. Toppoonyanont[2] employed flower buds as explants to induce cluster buds, and fewer studies have been conducted on the rapid proliferation of 'Kimono Rose'. The study utilized *C. alismatifolia* 'Kimono Rose' as the research object, selected the superior explant parts through sterilization and inoculation of four explant portions, and conducted a comparative analysis of the impact of varying concentrations of hormones on its induction and proliferation. This was done with the objective of enhancing the industrialized breeding system of tissue culture propagation of *C. alismatifolia* and integrating technology and production practice, while meeting the market demand for the seedling production of *C. alismatifolia* 'Kimono Rose'.

2 Materials and methods

2.1 Materials The test materials were planted in the ginger germplasm resource nursery of the Fujian Institute of Tropical Crops. Seedballs with full shape and no damage, as well as plants with strong growth potential and no disease, were selected. The seedballs, lateral buds, young flower buds, and young leaves were taken for the establishment of *C. alismatifolia* explants. The plant should be as dry as possible prior to the sampling, as this would reduce the quantity of bacteria in the smaple.

2.2 Disinfection of explants

2.2.1 Disinfection of seedball. The seedball of *C. alismatifolia* should be soaked in washing powder water for 10 min, then gently agitated and rinsed with tap water in order to remove the washing powder water. Following a 30-min immersion in running water, the seedball should be transferred to an ultra-clean bench and disinfected with 75% alcohol for 50 sec. Subsequently, the seedball was disinfected with a 0.1% mercuric chloride solution containing one drop of Tween 80 for 15 min. Following five rinses with aseptic water, the material was cut into small pieces, retaining one or two bud points.

2.2.2 Disinfection of lateral buds. As the lateral buds, which constituted the main bud, underwent flowering, the new buds that emerged alongside them were excised and rinsed under running water for 30 min. They were then sterilized with 75% alcohol for 50 sec, and subsequently disinfected with a 0.1% mercuric chloride solution containing one drop of Tween 80 for 10 min. Subsequently, the material was rinsed five times with aseptic water and then cut into small pieces with bud points corresponding to bud size.

2.2.3 Disinfection of young flower buds. The immature inflores-

Received: March 20, 2024 Accepted: July 16, 2024

Supported by Special Project of Public-interest Scientific Institutions of Fujian Province (2021R1011003).

* Corresponding author. E-mail: hexuej007@126.com

cences that had not yet blossomed were removed, and the young flower buds were excised by peeling off the leaf sheaths on a super-clean bench. The surface was disinfected with 75% alcohol for 50 sec, followed by a 1-min disinfection with a 0.1% mercuric chloride solution containing one drop of Tween 80. Subsequently, the flower buds were inoculated following five rinses with aseptic water.

2.2.4 Disinfection of young leaves. The leaves that had not yet developed were selected, disinfected with 75% alcohol for 30 sec, and then disinfected with a 0.1% mercuric chloride solution containing one drop of Tween 80 for 6 min. Subsequently, the young leaves were rinsed five times with aseptic water and then cut into small pieces measuring approximately 0.5 cm × 1 cm, after which they were inoculated onto the medium.

2.3 Induction of cluster buds The fundamental medium utilized for the induction of cluster buds was MS, combined with 6-BA and NAA. The concentrations of 6-BA were set at 0, 1, 3, 5, and 10 mg/L, while the concentration of NAA was fixed at 0.2 mg/L. Sucrose was added at a concentration of 25 g/L, agar at 6.5 g/L, and the pH was adjusted to a range of 5.8 – 6.0. To prevent cross-infection, only a single explant was introduced to each bottle. Following inoculation, the medium was changed at one-month intervals, and the contamination rate was recorded. During this period, browning tissues and leaves were excised as necessary, small shoots were retained, and the number of explant shoots initiated in the first month after inoculation was recorded. Contamination rate = Number of contaminated explants/Number of inoculated explants × 100%; Bud initiation rate = Number of buds initiated by explants/Number of non-contaminated explants ×100%.

2.4 Proliferation of cluster buds The fundamental medium for the proliferation of cluster buds was MS, combined with 6-BA and NAA. The concentration of 6-BA was set at 0, 1, 3, 5, and 10 mg/L, while the concentration of NAA was 0.1 mg/L. Sucrose was present at a concentration of 25 g/L, agar at 6.5 g/L, and the pH was maintained between 5.8 and 6.0. The healthy buds were selected from the induced cluster buds, the leaves and roots were removed, and the plant material was cut into small pieces with one to two small buds. Each bottle was inoculated with three buds. The number of emerged buds after inoculation was recorded, and the proliferation coefficient was calculated. Proliferation coefficient = Number of buds after subculture/Number of buds before subculture.

2.5 Data processing All data were statistically analyzed using Excel and SPSS.

3 Results and analysis

3.1 Comparison of explants The outcomes of the induction of cluster buds for disparate explants are presented in Table 1. The data demonstrated that the contamination rate varied significantly between the four explant types. Notably, the seedball contamination rate was the highest, indicating that the seedball itself carried a substantial bacterial load. The contamination rates of the explants from the remaining three parts were lower, with all values below 20%. The contamination rate of the young leaves was the lowest, yet it was unable to induce cluster buds. Although the young flower buds had the potential to induce cluster buds, their initiation rate was low, and their growth was slow. This made them well-suited for germplasm preservation but less suitable for production. The lateral buds exhibited a low contamination rate and a high clustering rate of cluster buds, and the inoculation procedure was relatively straightforward, rendering them a superior material for *C. alismatifolia* explants.

Table 1 Contamination of different explants and induction of cluster buds

Explant	Contamination rate//%	Initiation rate of cluster buds//%	Inoculation status
Seedball	46.33	45.32	Susceptible to contamination, susceptible to damage, more difficult inoculation operation
Lateral buds	19.67	78.30	Less contamination, easy bud formation, relatively simple inoculation operation
Young flower buds	14.67	12.61	Less contamination, but slower growth, and more complicated inoculation operation
Young leaves	2.00	0.00	Less contamination, but no sprouting

3.2 Screening of induction medium The outcomes of the investigation into the optimal induction medium for *C. alismatifolia*, primarily through the examination of varying concentrations of 6-BA, are presented in Table 2. It was observed that when the concentration of 6-BA was increased to 10 mg/L for young leaves, no indications of sprouting were exhibited. This suggested that it was challenging to induce cluster buds as an explant. The initiation rate of cluster buds exhibited a tendency to increase with the elevation of the 6-BA concentration for the remaining three explants. The number of cluster buds was observed to be higher when the concentration of 6-BA was 10 mg/L. Nevertheless, the buds displayed a deficiency in vigor, a light green hue, and a slender morphology. Upon subculturing, the growth of the seedlings exhibited a diminished vigor. It was challenging to induce the formation of cluster buds in young flower buds when using a low concentration of 6-BA as the inducing agent. When the concentration of 6-BA reached 5 mg/L or above, it was possible to induce the conversion of flower buds into leaf buds, and even cluster buds, yet the process was complex and growth was relatively slow. Both seedballs and lateral buds demonstrated the capacity to induce cluster buds. The induction rate of lateral buds was observed to be higher than that of seedballs. The cluster bud initiation rate of lateral buds reached 82.52% when the concentration of 6-BA was 5 mg/L. Therefore, cluster buds were induced by superior explant lateral buds, and the optimal medium was MS + 6-BA 5 mg/L + NAA 0.1 mg/L + sucrose 25 g/L + agar 6.5 g/L, with a pH range of 5.8 – 6.0.

Table 2 Impact of varying concentrations of 6-BA on induction of *Curcuma alismatifolia*

Explant	6-BA concentration // mg/L	Initiation rate of cluster buds // %
Seedball	0	32.71
	1	49.36
	3	50.00
	5	52.37
	10	54.92
Lateral buds	0	37.04
	1	71.33
	3	75.36
	5	82.52
	10	79.67
Young flower buds	0	0
	1	0
	3	0
	5	12.37
	10	30.51
Young leaves	0	0
	1	0
	3	0
	5	0
	10	0

3.3 Screening of proliferation medium The cluster buds induced by lateral buds were cultured to investigate the impact of varying concentrations of 6-BA on their proliferation, as illustrated in Table 3. As the concentration of 6-BA increased, the proliferation coefficient also increased. At a 6-BA concentration of 5 mg/L, the proliferation coefficient attained its maximum value of 2.84. However, the proliferation process revealed that an elevated concentration of 6-BA exerted an inhibitory effect on the elongation and growth of new buds, facilitating the formation of bud clusters, which was unfavorable for industrialized, orderly seedling cultivation. Nevertheless, at a 6-BA concentration of 3 mg/L, a high proliferation coefficient was observed. It was thus determined that the optimal medium for the growth of cluster buds conducive to the production of industrialized seedlings was MS + 6-BA 3 mg/L + NAA 0.1 mg/L + sucrose 25 g/L + AGAR 6.5 g/L, with the pH adjusted to a range of 5.8 – 6.0.

Table 3 Impact of varying concentrations of 6-BA on proliferation of *Curcuma alismatifolia*

Explant	6-BA concentration // mg/L	Proliferation coefficient
Lateral buds	0	1.39
	1	1.62
	3	2.73
	5	2.84
	10	1.42

4 Discussion

Tissue culture seedling production can be achieved through two principal organ pathways: direct and indirect[3]. The present study demonstrated that it was challenging to induce cluster buds using young leaves of *C. alismatifolia*. This finding suggests that the indirect organogenetic pathway may not be a viable approach for tissue culture and rapid propagation of *C. alismatifolia*. Additionally, the study revealed that this plant was among those exhibiting difficulty in inducing callus and redifferentiation. Similarly, Mahadtanapuk *et al.*[4] obtained comparable outcomes. The lateral bud represents a direct organogenetic pathway, offering the advantages of a low mutation rate and good maternal character, which are of significant benefit to seedling production. The utilization of seedballs as explants has been observed to result in a suboptimal efficacy of sterilization, which in turn has been identified as a significant contributing factor to the elevated incidence of contamination. Additionally, the buds carried on the seedballs are relatively young and susceptible to sterilization, which is not conducive to mass production. Although young flower buds have a relatively low contamination rate, they require sufficient hormone levels to complete the initiation of cluster buds. Additionally, they exhibit reduced growth rates, which makes them less desirable as propagation material for outgrowths.

MS has been extensively utilized in the establishment of a tissue culture system for *Curcuma*, as previously documented in the literature[5-7]. This system was also employed in the present study. The use of plant growth regulators in conjunction with the tissue culture process resulted in the robust growth of *C. alismatifolia* tissue culture seedlings, thereby enhancing the quality of the tissue culture results. The plant growth regulator 6-BA was employed in both the induction and proliferation of cluster buds, demonstrating efficacy in the induction of cluster buds when the concentration of 6-BA was high. However, excessive 6-BA resulted in the inhibition of elongation and the emergence of a fascicled state during proliferation.

References

[1] MOU XL, LI WJ, WANG JH, *et al.* Tissue culture and rapid propagation of *Curcuma alismatifolia*[J]. Northern Horticulture, 2006(5): 23. (in Chinese).

[2] TOPPOONYANONT N, CHONGSANG S, CHUJAN S. Micropropagation scheme of *Curcuma alismatifolia* Gagnep[J]. Acta Horticulturae, 2005(673): 705 – 712.

[3] LIU XM, LUO XM, WANG Z, *et al.* Micro propagation system establishment of *Zelkova schneideriana* through a direct organogenesis way[J]. Journal of Natural Science of Hunan Normal University, 2014, 37(2): 17 – 21. (in Chinese).

[4] MAHADTANAPUK S, TOPPOONYANONT N, HANDA T. Genetic transformation of *Curcuma alismatifolia* Gagnep. using retarded shoots[J]. Plant Biotechnology, 2006, 23(2): 233 – 237.

[5] COUSINS MM, ADELBERG JW. Short-term and long-term time course studies of turmeric (*Curcuma longa* L.) microrhizome development *in vitro*[J]. Plant Cell, Tissue and Organ Culture, 2008, 93(3): 283 – 293.

[6] HASHEMY T, MAKI H, YAMADA Y. Effects of light and cytokinin on *in vitro* micropropagation and microrhizome production in turmeric (*Curcuma longa* L.)[J]. Plant Biotechnology, 2009, 26(2): 237 – 242.

[7] JIE EY, AHN MS, LEE J, *et al.* Establishment of a high-frequency plant regeneration system from rhizome-derived embryogenic cell-suspension cultures of *Curcuma longa* L.[J]. Plant Biotechnology Reports, 2019(13): 123 – 129.