# Rapid Propagation System in Vitro of Medicinal Plant *Cynanchum Atratum* Bunge

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**Research Article**

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# Abstract

As a traditional Chinese medicinal material, *Cynanchum atratum* Bunge has been widely used in traditional Chinese medicine for its treatment of abscesses, acute urinary infection and hectic fever.Thus, wild resources of it have been endangered by overharvesting. Plant tissue culture technology is an important measure to protect wild resources of medicinal plants, including *C. atratum*. Therefore, a fast and efficient propagation system of *C. atratum* through axillary bud proliferation pathwayhas been established. Through axillary bud proliferation, the medium [MS+sucrose 30 g/L+Agar 7 g/L+NAA 0.2 mg/l+IBA 1.5mg/l+KT 0.5 mg/l] can effectively proliferate adventitious buds, and the induction rate was 100 %, proliferation coefficient could reach 8.56. MS medium was used to induce adventitious bud rooting, with rooting rate of 98% and no callus. The highest survival rate was 90% when the ratio of grass mud pond and orchard red soil was 1:1. To our knowledge this is the first report of rapid propagation system in *C. atratum*, it achieve rapid reproduction of *C. atratum*.


# Main Text

*Cynanchum atratum* Bunge is an erect perennial dicotyledonous herb of Asclepiadaceae *Cynanchum atratum*, commonly called ‘baiwei’ , ‘Weicao’ and ‘Laojunxu’ in Chinese.(De Z, Ya-Ling L 2010). The dry stem and root of *C. atratum* are commonly used in traditional Chinese medicine, which have good antitussive, antiasthmatic, anti-inflammatory, and blood pressure lowering effects (Li 2006). Therefore, it is widely used in clinical antipyretic and modern pharmacological studies. In recent years, it has been found that C. atratum contains saponins, which can inhibit bacteria and inflammation, as well as volatile oil and cardiac glycosides. The chemical composition identification in previous studies was mainly focused on C21 steroidal saponins. Until now, approximately 440 compounds have been isolated from *C. atratum*, including C21 steroidal glycosides, steroidal saponins, alkaloids, flavonoids, and terpenoids (Qiu et al. 1991; Li et al. 2006; Lu et al. 2011; Gu and Hao. 2016; Choi et al. 2017).

Due to the continuous research and development of pharmacological effects and components of *C. atratum*, its medicinal value has been gradually excavated, which resulted in a large number of wild *C. atratum* being lost due to seeds reduced.Over harvesting of *C. atratum* caused a threat to its sustainable development. Therefore, artificial domestication cultivation and seedling production of *C. atratum* are of importance. In recent years, with the improvement of biotechnology theory and experimental condition , endangered medicinal plants can be protected by plant tissue culture technology. Excellent results have been achieved in the field of rapid propagation system of medicinal plants worldwide (Lucchesini M et al. 2010; Siddique et al. 2010; Aremu et al. 2013; Monemi et al. 2014; Gupta et al. 2014). According to statistics, in Europe and the United States plant tissue culture technology has been widely used in seedling mass production; In China, the rapid propagation system of more than 200 medicinal plants has been established. The growing trend of rapid propagation system of medicinal plants provides reference for the research and protection of medicinal plant resources (Mercier, H et al. 1992), but there are no reports on the rapid propagation system of *C. atratum*.

Here we set up a fast and efficient propagation system of *C. atratum* which is of great significance to the protection of wild resources and seedling production of *C. atratum*.

The seeds of *C. atratum* were soaked in 45°C water for 3 h, sterilized with 75% alcohol for 30 s, then the seeds were soaked in 0.1% mercuric chloride ($HgCl_2$) for 12 min. and rinsed with sterile water for 3~5 times. Then the seeds were germinated on 1/2 MS (Murashige and Skoog, 1962) medium. The cultures were moved in culture room at 25±2°C and kept under 2500 lx of light intensity with 14 h light period. Explants of 1-1.5 cm length from stem segments of 4-week-old seedlings were cultured on MS medium containing NAA (0.2 mg/l, 2 mg/l), IBA (0.1-2 mg/l), and KT (0-2 mg/l). Single buds of 2-5 cm were isolated and tranlplanted in the medium containing different concentrations of NAA (0-1 mg/l) for rooting culture after 3 weeks. When the root length of seedlings reached 3-5 cm, open the bottle cap, and water of 1-2 cm was injected into the bottle. After 3-5 days, the plant was removed from the culture medium and washed gently to remove the adhered medium. Then the root portion was treated with 0.1% carbendazim for a moment. After treatment, the seedlings were transplanted into the substrate of humus: orchard red soil = 1:1.

Explants of 1.0–1.5 cm length from stem segments of 4-week-old seedlings can successfully proliferate adventitious buds from Medium 3-6, the best effect of adventitious bud proliferation was No. 4 (MS+NAA 0.2 mg/l+IBA 1.5 mg/l+KT 0.5 mg/l), with 8.56 buds per explant (table 1). while No. 1 and No. 2 had no significant effect on adventitious shoot proliferation. After 20 days, the rooting effects of tissue culture seedlings under different treatments were recorded and compared. Among the seven treatments for rooting, MS medium without any plant growth regulator had the best rooting effect (No.11), the rooting rate was 98%, and the root state was the best (table 2). The domesticated seedlings were cultured in laboratory condition for 2 weeks and then transferred to outdoor. After 4 weeks, the seedlings were transplanted successfully, and the survival rates were 90%.

The rapid development of plant tissue culture technology provides a new opportunity for the protection and sustainable development of many endangered plant resources, and realizes the rapid production of seedlings. At present, important breakthroughs have been made in the research on tissue culture technology of Asclepiadaceae plants, and this technology is also constantly improving. In addition, excellent results have been obtained in the establishment of rapid propagation system of *Cynanchum bungei* Decne, which is the same genus of *C. atratum*, and the rapid propagation and industrial production of *C. bungei* have been realized (Wen-Liang D. 2005; Wen-Liang D et al. 2008; Xun J et al. 2010). In previous studies, Wen-Liang D found that the best medium for axillary bud proliferation of *C. bungei* was MS+6-BA 0.20 mg/L+NAA 0.05 mg/L, and Han-guang W found that the best medium for axillary bud proliferation of *C. bungei* was MS+6-BA 0.20 mg/L+NAA 0.10 mg/L, The results are similar. In this study, we found that the best medium for axillary bud proliferation of *C. atratum* was MS+NAA 0.2 mg/L+IBA 1.5 mg/L+KT 0.5 mg/L, which was different from the *C. bungei*. The concentrations required for various plant growth regulators to achieve the best effect are different, which may be that the sensitivity of *C. atratum* to various plant growth regulators is different. When the concentrations of NAA and KT are too high, it is not conducive to the proliferation of adventitious buds of *C. atratum*. Therefore,

the appropriate combination of auxin and cytokinin is more conducive to the proliferation of adventitious buds of *C. atratum*. Although NAA can induce rooting of *C. atratum*, the roots induced by NAA are short and accompanied by callus formation, which is not conducive to the growth of tube seedlings, and the results are the same as those of Rui-Zhi P et al (Rui-Zhi P et al. 1995). The results showed that the rooting rate was 98 % on MS medium without any phytohormones, so the best rooting medium was MS.

Transplanting medium is the carrier of nutrients and water for tissue culture seedlings, and it is also the microenvironment for root growth. Ventilation, water holding capacity and nutrients of transplanting medium are important factors affecting the survival of tissue culture seedlings(Lei G et al. 2006; Hai-Xia C et al. 2017). *C. atratum* likes good drainage, fertile, deep soil layer and humus-rich loam. When the volume of humus and orchard red soil was mixed at 1:1, the substrate had good water holding capacity, high permeability, and the survival rate was the highest, reaching 90%, which was the best transplanting medium for *C. atratum*.

At present, with the development of *C. atratum* resources, the market demand for *C. atratum* is increasing, while the supply and demand of *C. atratum* are limited, and the cultivation technology of *C. atratum* is immature, which makes the protection of *C. atratum* resources face severe challenges. At present, there are few reports on the rapid propagation system of *C. atratum*. Through the proliferation and rooting of axillary buds of *C. atratum*, the technical difficulties in large-scale cloning production of *C. atratum* test-tube seedlings are effectively solved.

## Declarations

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# Tables

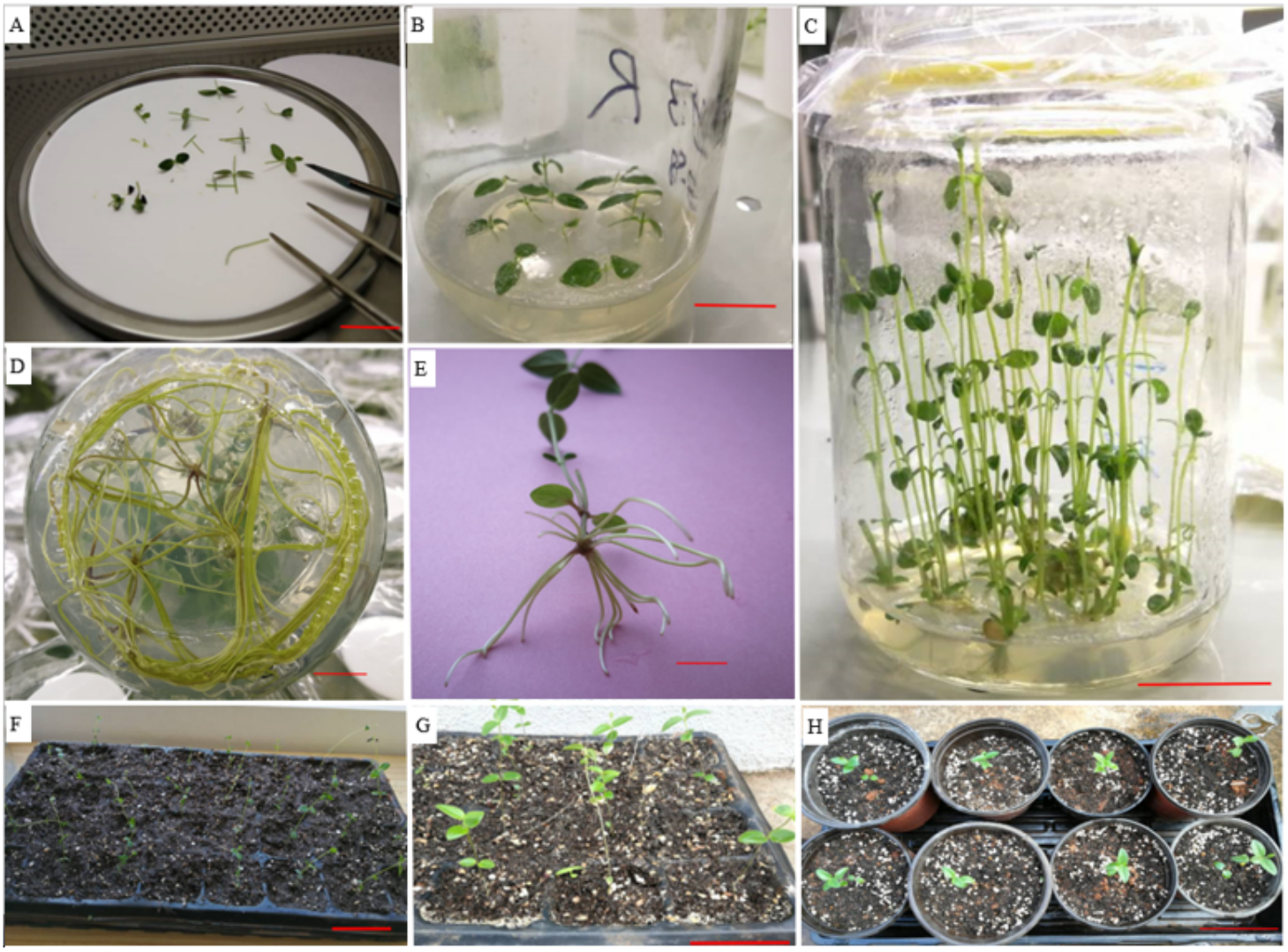
**Table 1 Adventitious bud proliferation of *C. atratum***

| No. | primary medium | Plant growth regulator concentration(mg/l) | | | Number of explants | proliferation coefficient | Proliferation rate (%) |
|---|---|---|---|---|---|---|---|
| | | NAA | IBA | KT | | | |
| 1 | MS | 2 | 2 | 0 | 50 | 0 | 0 |
| 2 | | 2 | 1 | 0.5 | 50 | 0 | 0 |
| 3 | | 2 | 0.1 | 2 | 50 | 1.05 | 105 |
| 4 | | 0.2 | 1.5 | 0.5 | 50 | 8.56 | 856 |
| 5 | | 0.2 | 1.5 | 1 | 50 | 2.34 | 234 |
| 6 | | 0.2 | 1.5 | 2 | 50 | 3.15 | 315 |

**Table 2 Effects of different hormone concentrations on the rooting of *C. atratum***

| No. | primary medium | NAA(mg/l) | Number of rooting | Number of explants | Rooting rate (%) | Characteristics of roots |
|---|---|---|---|---|---|---|
| 7 | MS | 0.1 | 70 | 100 | 70 | Fine and long, little callus |
| 8 | | 0.5 | 57 | 100 | 57 | Fine and long, little callus |
| 9 | | 0.8 | 34 | 100 | 34 | Thick and short, little callus |
| 10 | | 1 | 20 | 100 | 20 | Thick and short, little callus |
| 11 | | 0 | 98 | 100 | 98 | Thick and long, no callus |
| 12 | 1/3MS | 0 | 80 | 100 | 80 | Fine and long , no callus |
| 13 | 1/2MS | 0 | 85 | 100 | 85 | Thick and long, no callus, but the color is red |

# Figures




## Figure 1

Three Stages in the development of rapid propagation system of C. atratum A: Stem segments cut from sterile seedlings; B: The stem segments with axillary buds growing in No.4 Medium; C: Adventitious bud proliferating and growing for 30 days. Bar = 2 cm. D: Roots normally grown in medium; E: Seedlings with normal rooting. Bar = 1 cm F: Domestication of Seedlings under laboratory condition; G: Seedlings domesticated for 2 weeks; H: Plants growing normally in the pot. Bar = 10 cm