广 西 植 物 Guihaia 26(3):286 — 288 2006 年 5 月

Rapid plant regeneration from *Glechoma longituba*

HAN Su-ju, LI Yun-xiang*, YANG Zi-song,
JIANG Tian-liang, LI You

(*Sichuan Provincial Key Laboratory of Environmental Science and Biological Diversity Conservation, China West Normal University*, Nanchong 637002, China)

Abstract: A successful micropropagation technique of *Glechoma longituba* (Nakai) Kupr was established from the explants of auxiliary buds and stem-tips. The result showed that: (1) MS medium with 0.1 mg/L NAA and 1.5 mg/L BA could promote the growth of vegetative auxiliary buds and regeneration of plentiful lateral buds. (2) Shoots derived from auxiliary and lateral buds could form roots on MS medium with 1.0 mg/L IBA and 1.0 mg/L KT, the proportion was up to 100%.

Key words: *Glechoma longituba*; tissue culture; rapid propagation

CLC number: Q943.1 **Document code**: A **Article ID**: 1000-3142(2006)03-0286-03

Glechoma longituba (Nakai) Kupr is an important medicinal herb (Chinese Pharmacopoqia, 2000). In the traditional system of Chinese medicine, *G. longituba* is a reputed medicinal herb and contains lots of useful chemical compositions in its leaves, stems and roots such as flavonoids, ursolic acid, choline and so on (Wang, *et al.*, 1995; Jin, 2001). At the moment, the wild stock has been markedly depleted because pharmaceutical companies largely depend upon material procured from naturally occurring stands, which are being depleted rapidly raising concern about possible extinction of the species and providing justification for the development of *in vitro* propagation techniques for plant regeneration from stem-tip and auxiliary buds of *G. longituba*. In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal herbs (Li, 2003). Since 20th century, the tissue culture and the fast reproduction technology already widely have applied in each kind of *Labiatae* medicinal plant (Duan *et al.*, 2001) Therefore, it is important to develop an efficient micropropagation technique for *G. longituba* to rapidly disseminate superior clones once they are identified. Tissue culture technique can play an important role in the propagation of *G. longituba* and the elite herb conservation of *G. longituba*. In addition, compounds from tissue culture may be more easily purified because of simple extraction procedures to possibly reduce the production and processing costs.

1 Materials and methods

1.1 Plant material

Auxiliary buds and shoot-tips were collected from *G. longituba* grown in greenhouse.

1.2 Experiment methods

1.2.1 Surface sterilization The materials were surface sterilized as follows: (1) Flushing for 30

收稿日期: 2005-07-26 **修回日期**: 2005-12-25

基金项目: 四川省杰出青年学科带头人培养计划项目(04ZQ026-047); 四川省科技厅基础项目(03JY029-021-22) [Supported by Outstanding Youth and Leader Cultivated Item(04ZQ026-047); Sichuan Science and Technology Basic Item of Sichuan (03JY029-021-22)]

作者简介: 韩素菊(1976-),女,四川资中人,硕士研究生,主要从事药用植物的有关研究。

* 通讯作者(Author for correspondence, E-mail: yx_li@263.net)

min under running tap water followed removal of leaves. Shoot tip pieces of about 1.5 cm were excised from the material; (2)Rinsing with 70% ethanol for 10 s; (3)Sterilizing with 2% Javel water for 30 seconds and washing with autoclaved sterilized distilled water for 5 min(4 times); (4) Trimming the explants to about 1.0 cm prior to inoculation in culture medium.

1.2.2 Culture medium　Explants were placed on solid basal MS medium with sucrose(3.0 g/L)and agar(0.8 g/L)supplemented with different concentrations and combination of BA(0.5,1.0,2.0,2.5, 3.0 mg/L)and NAA(0.1 mg/L)for the proliferation of explants. The pH value of the media was adjusted to 5.8 using 0.1 N HCl or 0.1 N NaOH before autoclaving. Media were sterilized at 121℃ and 104 kPa for 15 min. After thirty days, the proliferations of buds were re-divided and transferred onto another MS medium with 1.0 mg/L IBA and 1.0 mg/L KT to induce rooting and formation of complete plantlets.

1.2.3 Culture condition　All the cultures were maintained at(25±2)℃ with 12～14 hour's photoperiod in cool white fluorescent light(1 500～2 000 lx).

2　Result and discussion

2.1 Effects of sterilization

The sterilizing duration of explants had influences on the percentage of pollution. The results of observation and statistics after inoculated for 10 days can be found in table 1.

It was difficult to sterilize explants because of its dense epidermal hairs. Almost all of the explants were decontaminated after a short duration in Javel water. While explants were dipped in the Javel water for 10 s, the percentage of pollution was 80% and it was the highest. After sterilizing for 30 s, it was 10%. There was no obvious influence on the percentage while the explants were dipped for 30～50 s. However, the proliferation buds became thin and weak after explants were dipped in the Javel water for over 40 s. Considering the percentage of pollution and the quality of the proliferation, the best sterilization duration should be 30 s.

2.2 Proliferation of shoots

Explants were cultured on MS medium with different concentrations and combinations. There was an obvious sign with the alteration of hormones. The results were observed after being cultured for 30 days.

Table 1　Effects of the different sterilization duration on the percentages of contaminated explants

Sterilization duration(s)	Number of explants	Number of pollution	Percentage of pollution(%)
10	30	24	80.0
20	30	9	30.0
30	30	3	10.0
40	30	4	13.3
50	30	2	6.7

Table 2　Effects of different concentration of hormones on induction proliferation

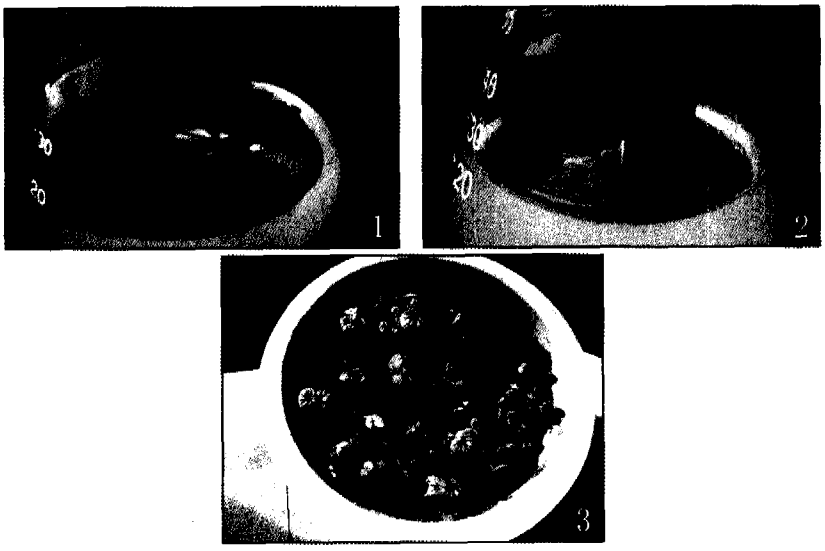
MS medium (mg/L)		Number of explants	Number of pollution	Number of proliferation buds	Proliferation times
BA	NAA				
0.5	0.1	30	3	56	2.07
1.0	0.1	30	2	142	5.07
2.0	0.1	30	2	197	7.04
2.5	0.1	30	3	289	10.70
3.0	0.1	30	4	341	13.12

Each segment formed 5～10 shoots by the proliferation of apical and axillary meristems. In Table 2, the different times of proliferation could be due to the varying concentrations of BA used in the medium. The times of proliferation increased with an increase in the concentration of BA and the rate varied from 2.07 to 13.12, so the higher concentration of BA was found to promote the proliferation on plantlets. However, the young shoots became thin, long and weak with increasing in concentrations. It makes the survival rate of transplant lower. Considering the rate of proliferation and the quality of plantlets, the proper concentration of BA should be 2.0 mg/L when inducing the proliferation of multiple shoots(Figs. 1-3; 1).

2.3 Root development in regenerated plants

The proliferation buds were separated and

transferred to the rooting medium to form complete plantlets. Roots were formed on young buds grown in MS medium containing IBA(1.0 mg/L) and KT(1.0 mg/L)(Figs. 1-3:2). A high percentage of shoots(100%)rooted in the medium. Root initially formed in 8～10 d and developed into a good root system in 12～16 d. Even roots were formed on MS medium without growth regulators. This may show that *G. longituba* has inherent hormone to promote itself to root.



Figs. 1-3 1. Buds of clone; 2. Roots of cloned plants; 3. Survival plants on nutritional soil.

2.4 Acclimatization of rooted plant

After being cultured for 15～20 d in the taking roots media, when the plantlets were 3～4 cm high, with 6～9 roots(2～3 cm long), the tubes were taken to the outdoor. Two days later, rooted plantlets were taken out of the tubes and washed off the medium. The plantlets were transferred into pots containing sand:humus in the ratio of 3∶1 and the pots were put under the shade. Ten days later, the plantlets grew well(Figs. 1-3:3).

In conclusion, we have developed the tissue culture system of an important medicinal herb of *G. longituba*. The results of this study will hopefully offer us opportunities not only for rapid and economical propagation of *G. longituba*, but also for its conservation of the species.

参考文献:

中华人民共和国卫生部药典委员会. 2000. 中华人民共和国药典[S]. 北京:化学工业出版社:134－135.

王修堂,田 野. 1995. 连钱草的药用价值与科学栽培[J]. 中草药,**26**(10):557.

李浚明. 2003. 植物组织培养教程[M]. 北京:中国农业大学出版社,**12**:32－34.

金淑琴. 2001. 金钱草广金钱草连钱草的考证及临床应用[J]. 中医中药,**8**(11):54.

Duan HJ(段黄金),Gao JS(高疆生),Zhao SZ(赵书珍),*et al*. 2001. A study on propagation of *Coleus blumei*(彩叶草的组培快繁研究)[J]. *Chin Agri Sci Bull*(中国农学通报),**17**(1):25－27.

连钱草组培快繁技术研究

韩素菊,黎云祥*,杨子松,姜天亮,李 尤

(西华师范大学 四川省环境科学与生物多样性保护重点实验室,四川 南充 637002)

摘 要:用连钱草无菌茎尖为外植体进行快速繁殖,分别诱导、分化、生根形成再生植株进行快速繁殖,并移栽成活。结果表明在MS+6-BA 1.5 mg/L+NAA 0.1 mg/L培养基上诱导丛生芽效果最佳。在MS+IBA 1.0 mg/l+KT 1.0 mg/L培养基中根的诱导率为100%。

关键词:连钱草;组织培养;快繁