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珍稀药用和观赏植物地涌金莲的组织培养和快速繁殖

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摘要：以地涌金莲(*Musella lasiocarpa*)吸芽为外植体建立了有效的快繁体系。外植体经灭菌处理后在 MS+6-BA 4.0 mg L^{-1}+NAA 0.2 mg L^{-1}+ 维生素 C 150 mg L^{-1}+10%椰子乳 +3%蔗糖培养基上能进行不定芽的诱导和增殖，培养 60 d 后每个芽平均能产生 4.10 个不定芽。第 6 代增殖后，丛生芽的增殖系数可达 4.23。生根培养基以 1/2MS+NAA 1.0 mg L^{-1}+AC 50 mg L^{-1} 的效果较好。以沙：泥炭土:珍珠岩 =1:1：1 为基质移栽试管苗，成活率达到 93.5%以上。经过 12 个月的组织培养已生产 10 000 多株试管苗。

关键词：地涌金莲；组织培养；微繁殖
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Tissue Culture and Rapid Propagation of *Musella lasiocarpa*, a Rare Chinese Medicinal and Ornamental Herb

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Abstract: An efficient and rapid micropropagation system by using auxiliary buds as explants has been established for *Musella lasiocarpa*. Murashige and Skoog (MS) medium supplemented with 4.0 mg L^{-1} 6-benzylaminopurine, 0.2 mg L^{-1} 1-napthaleneacetic acid, 150 mg L^{-1} vitamin C, 10% coconut milk and 3% sucrose was suitable for shoot induction and proliferation from divided buds. Each explant produced an average of 4.10 shoots after 60-day culture. After the sixth subculture, the shoot proliferation rate of plantlets was 4.23-fold. The half-strength MS medium supplemented with 1.0 mg L^{-1} indole-3-butyric acid and 150 mg L^{-1} activated charcoal was suitable for rooting. When micropropagated plantlets with well-developed root systems were transferred to planting bed containing a mixture of sand, sieved peat and perlite (1:1:1; v/v) in a greenhouse, 93.5% of the plantlets survived. About 10 000 plantlets were produced successfully for field transfer after 12 months of culture initiation. This production system is useful for *ex situ* conservation and large-scale multiplication of *M. lasiocarpa*.
Key words: *Musella lasiocarpa*; Tissue culture; Micropropagation

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Abbreviations: BA: 6-benzylaminopurine; NAA: Napthaleneacetic acid; CM: Coconut milk; IBA: Indole-3-butyric acid; AC: Activated charcoal; MS: Murashige and Skoog.

Musella lasiocarpa (Franch.) C. Y. Wu ex H. W. Li is native primarily to Southwestern China and has been used for several centuries as "Ngay-tsiao"(rock banana) [1-2], "Di-Yun-Jin-Lian" (Golden lotus rising from the ground)[3]. *M. lasiocarpa* is a large, perennial herb with rhizome, mainly used as medicine, food, and fodder [4-6]. In medicinal use, the fresh flowers and bracts are mashed into plaster and applied externally to stop bleeding and to counteract inflammation of body skin. An infusion of young inflorescences is boiled and taken internally to treat enteritis, constipation, and female diseases. The sap is also taken internally to detoxify monkshood poisoning and to alleviate drunkenness. In addition, the inner pseudostem is edible and can be made into different types of food after removal of the leaf sheaths and the leaves, while pseudostem and rhizome are used as fodder for pigs and their tough fibers are used to make ropes, belts and chairs. *M. lasiocarpa* blooms in all year and is valued as an ornamental garden plant because of its long-lasting, bright yellow inflorescences[7].

In nature, *M. lasiocarpa* can be propagated by seeds, auxiliary buds or rhizomes; however, the germination of seeds needs 1–2 years and the vegetative propagations of auxiliary buds and rhizomes are so slow for large-scale production. Thus, conventional propagation through seeds, auxiliary buds or rhizomes can't sufficiently meet the practical demand. On the other hand, the application of tissue techniques might allow rapid and large-scale propagation of uniform plants. Therefore, *in vitro* micropropagation of this plant will have great importance for germplasm conservation and commercial production. To authors' best knowledge, there is only a brief communication report of regeneration of *M. lasiocarpa* through tissue culture[8]. In this paper, we established a system for high-frequency *in vitro* multiplication of *M. lasiocarpa* by auxillary buds for the first time. The role of salt concentration, plant growth regulators, activated charcoal, coconut milk and vitamin C on propagation or rooting were evaluated in the study.

1 Materials and methods

1.1 Plant materials and explant decontamination

M. lasiocarpa plants collected from the natural habitat (Yunnan), were maintained at the South China Botanical Garden, the Chinese Academy of Sciences. Shoot explants were obtained from the auxiliary buds of vigorous plants bearing inflorescences grown in greenhouse or in the field (Plate I: A). The auxiliary buds with leaf bases removed were washed in running tap water for 15 min, then immersed in 70% ethanol for 30 s followed in 0.1% (w/v) $HgCl_2$ solution with continual agitation for 10 min, and finally rinsed 4 times with sterile distilled water. The tissues of each bud were further trimmed to about 1 cm^3, sterilized with 1% sodium hypochlorite (NaOCl) solution for 5 min and then rinsed 5 times with sterile distilled water. Inoculated explant was undivided (Plate I: B) or longitudinally divided into2 pieces (Plate I: C).

1.2 *In vitro* plantlet production

The sterilized explants were cultured on Murashige and Skoog basal medium[9], supplemented with various concentrations (2%, 3% and 4%) (w/v) of sucrose, and solidified with 7 mg L^{-1} agar. A single bud including the bud longitudinally divided into 2 pieces was vertically placed in a 200 ml culture container. The medium was dispensed into a culture container with 30 ml medium supplemented with 6-BA (0, 1.0, 2.0, 4.0 and 6.0 mg L^{-1}) in combination with NAA (0, 0.2 and 0.5 mg L^{-1}) (Table 2). The culture container was closed with a plastic cap.

At the initial stage, the explant was cultured individually for 60 days. In order to reduce browning, 150 mg L^{-1} activated charcoal or 150 mg L^{-1} vitamin C was added into the medium, or the explant was transplanted onto the same fresh medium every 10 days (Table 1). Thereafter, the auxiliary buds from the culture were periodically subcultured every 40 days in the same fresh medium to obtain high multiplication rate. A cluster of 3 shoots was used as a unit of

propagation; five clusters of shoots were cultured in every culture container. The production rate (fold) of plantlets was calculated by dividing number of plantlets after 60-d culture from the sixth subculture.

1.3 Rooting

After the sixth subculture, the auxiliary buds were divided into single shoot inoculated on full or half-strength macroelement MS medium supplemented with different concentrations of IBA (0, 0.5, 1.0 and 2.0 mg L^{-1}) or NAA (0, 0.5, 1.0 and 2.0 mg L^{-1}). Five shoots were cultured together in a culture container.

1.4 Transfer of *in vitro* propagated plantlets to soil

Micropropagated plantlets with well-developed root systems were transferred to regular laboratory conditions for acclimatization with the container open for 3 days before the plantlets were transferred to planting bed. Then, the plantlets were taken out, and washed with running tap water to move the medium from the roots. The plantlets were transplanted to planting beds containing a mixture of sand, sieved peat and perlite (1:1:1; v/v) in the greenhouse with double layers of high-density polythene black agronet. The planting beds were covered with polythene pellicle to maintain a high relative humidity of 70%–80% under periodic mist for the first weeks. Polythene covers were removed gradually within 2 weeks. Two weeks later, the plantlets were fertilized with 0.1% hyponex 20-20-20 once in a week to enhance growth and development. The surviving rate was recorded in 4 weeks after transplant. The surviving plantlets were transplanted into plastic pots after 2 months to be acclimatized to the outside environment.

1.5 Culture conditions and statistical analysis

The MS medium supplemented with different concentrations of sucrose, 6-BA, NAA and IBA was used for *in vitro* cultures, prior to the addition of 150 mg L^{-1} vitamin C, 150 mg L^{-1} activated charcoal, and 10% coconut milk. The coconut milk used in these experiments was obtained from 6- to 7-month-old green coconuts. The milk was filtered through one sheet of filter paper. The medium were adjusted to pH 5.8 with 1 mol/L NaOH and 1 mol/L HCl before autoclave at 121℃ for 20 min at 1.06 kg cm^{-2}. The cultures were incubated at 25±2℃ with a 16-h photoperiod under a fluorescent light of approximate 45 μmol $m^{-2}s^{-1}$.

Each experiment was repeated three times and ten replications per treatment. All experiments were carried out in a completely randomized design. Data were subjected to ANOVA, and the level of least significance difference (LSD) was determined using Duncan's multiple range test at $P \leqslant 0.05$ for comparing means in the treatments[10].

2 Results

2.1 Shoot initiation and multiplication

All explants of *in vitro* culture produced exudation of phenolics from the cut ends and browned severely surrounding medium during the first weeks. However, 90% of explants from greenhouse and 83.3% of those from field survived (no bacterial and fungal contaminations or death) in shoot induction. Survived explants could product auxiliary buds within 30 days of culture.

On MS medium supplemented with 4.0 mg L^{-1} 6-BA and 0.2 mg L^{-1} NAA, few cluster shoots could be induced from the explants within 60 days because of severe browning. The above medium in combination with 150 mg L^{-1} activated charcoal (Plate I: D) or 150 mg L^{-1} vitamin C, or transplanting explants every 10 days onto the same fresh medium could reduce browning and increase the number of cluster shoots (Table 1). The incubation of *M. lasiocarpa* bud explants on these medium for 60 days resulted in 1.20–2.43 shoots for undivided explants, and 1.53–3.23 shoots for divided explants per explant. The number of auxiliary shoots from undivided explants or divided explants was significantly different at 5% level according to Duncan's Multiple Range Test in the same medium or culture ways (Table 1). On MS medium supplemented with 4.0 mg L^{-1} 6-BA,

0.5 mg L^{-1} NAA, and 150 mg L^{-1} vitamin C, there was the highest shoot proliferation rate (3.23 shoots per explant) with divided explants. While supplemented with 150 mg L^{-1} activated charcoal, the auxiliary buds induced with adventitious roots could be observed.

Addition of 3% of sucrose was more effective for sprouting of auxiliary buds than 2% or 4% of sucrose in shoot initiation (data not shown).

Table 1 Effects of medium and different culture ways on auxiliary shoot initiation and the length of *M. lasiocarpa* on MS medium containing 4.0 mg L^{-1} 6-BA, 0.2 mg L^{-1} NAA and 3% sucrose

Treatment	Undivided bud explants		Divided bud explants	
	Number of shoot inducted	Shoot length (cm)	Number of shoot inducted	Shoot length (cm)
Transplanted every 30 days (Control)	1.20 ± 0.61^{c}	2.88 ± 0.29^{d}	$1.53\pm0.57^{c*}$	$2.61\pm0.24^{c*}$
Transplanted every 10 days	2.27 ± 0.58^{a}	4.63 ± 0.37^{a}	$3.17\pm0.53^{a*}$	$4.13\pm0.37^{a*}$
MS + 150 mg L^{-1} vitamin C	2.43 ± 0.57^{a}	4.36 ± 0.23^{b}	$3.23\pm0.63^{a*}$	4.23 ± 0.62^{a}
MS + 150 mg L^{-1} AC	1.50 ± 0.5^{b}	4.03 ± 0.28^{c}	$2.17\pm0.59^{b*}$	$3.56\pm0.25^{b*}$

Values followed by different letters within a column are significantly different at 5% level by Duncan's Multiple Range Test. Thirty replicates were used in each treatment. Values marked with * are significantly different at 5% level between undivided and divided bud explants. Data were recorded after 60-day culture on initiation medium.

During the shoot initiation culture and subculture, the effect of combination with 6-BA, NAA and coconut milk on shoot induction and auxiliary shoot multiplication is shown in Table 2. The concentrations of growth regulators in the medium influenced markedly on shoot regeneration. When no plant growth regulator was used, there were few regenerated shoots. However, application of 6-BA 4.0 mg L^{-1}, NAA 0.2 mg L^{-1} plus 10% coconut milk was the most effective for shoot induction and auxiliary bud multiplication. The shoot proliferation rate was 4.10 in shoot induction and 4.23 in auxiliary

Table 2 Effects of various medium on auxiliary shoot initiation and the length of *M. lasiocarpa*

6-BA (mg L^{-1})	NAA (mg L^{-1})	0% Coconut milk				10% Coconut milk			
		Initiation		Subculture		Initiation		Subculture	
		Number	Length (cm)	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)
0	0	0.63 ± 0.49^{f}	1.87 ± 0.46^{g}	0.43 ± 0.50^{g}	2.52 ± 0.30^{g}	$1.80\pm0.55^{f*}$	$2.48\pm0.31^{h*}$	$1.13\pm0.43^{h*}$	$3.41\pm0.48^{ef*}$
1.0	0	1.27 ± 0.52^{e}	2.66 ± 0.36^{f}	1.50 ± 0.57^{f}	3.54 ± 0.46^{ab}	$2.53\pm0.86^{de*}$	$3.04\pm0.36^{g*}$	$1.93\pm0.52^{g*}$	$3.83\pm0.38^{ab*}$
2.0	0	2.33 ± 0.61^{d}	3.04 ± 0.30^{e}	1.83 ± 0.59^{ef}	3.30 ± 0.31^{c}	$3.27\pm0.74^{c*}$	3.22 ± 0.43^{fg}	$2.63\pm0.61^{ef*}$	$3.64\pm0.32^{bcd*}$
4.0	0	2.80 ± 0.55^{bc}	3.57 ± 0.23^{d}	2.63 ± 0.56^{c}	3.10 ± 0.30^{e}	$3.43\pm0.68^{bc*}$	$3.93\pm0.40^{cd*}$	$3.20\pm0.48*^{c}$	$3.44\pm0.35^{de*}$
6.0	0	2.63 ± 0.67^{ed}	2.68 ± 0.59^{f}	2.33 ± 0.66^{cd}	2.83 ± 0.33^{f}	2.80 ± 0.61^{d}	$3.51\pm0.41^{e*}$	2.43 ± 0.50^{f}	$3.03\pm0.32^{g*}$
1.0	0.2	1.47 ± 0.63^{e}	4.43 ± 0.59^{a}	1.70 ± 0.60^{f}	3.63 ± 0.38^{a}	$2.43\pm0.68^{e*}$	$5.06\pm0.48^{a*}$	1.90 ± 0.55^{g}	$4.09\pm0.41^{a*}$
2.0	0.2	2.77 ± 0.63^{c}	3.62 ± 0.28^{cd}	2.07 ± 0.64^{de}	3.29 ± 0.32^{c}	$3.23\pm0.63^{c*}$	$4.03\pm0.36^{cd*}$	$2.80\pm0.61^{de*}$	$3.85\pm0.32^{a*}$
4.0	0.2	3.23 ± 0.63^{a}	4.23 ± 0.62^{b}	3.63 ± 0.67^{a}	3.44 ± 0.29^{bc}	$4.10\pm0.71^{a*}$	$4.53\pm0.54^{b*}$	$4.23\pm0.57^{a*}$	$3.66\pm0.39^{abc*}$
6.0	0.2	3.07 ± 0.64^{ab}	3.53 ± 0.46^{d}	3.23 ± 0.77^{b}	3.05 ± 0.31^{e}	$3.53\pm0.63^{bc*}$	$4.12\pm0.31^{c*}$	3.07 ± 0.58^{cd}	3.22 ± 0.39^{f}
1.0	0.5	1.43 ± 0.57^{e}	4.20 ± 0.33^{b}	1.60 ± 0.62^{f}	3.47 ± 0.28^{abc}	$2.30\pm0.70^{e*}$	$3.89\pm0.23^{d*}$	$1.97\pm0.49^{g*}$	3.62 ± 0.44^{cd}
2.0	0.5	2.53 ± 0.68^{cd}	3.82 ± 0.38^{c}	2.03 ± 0.72^{de}	3.13 ± 0.38^{de}	$3.17\pm0.70^{c*}$	3.90 ± 0.35^{d}	$2.83\pm0.59^{de*}$	$3.30\pm0.26^{ef*}$
4.0	0.5	3.17 ± 0.55^{a}	4.12 ± 0.44^{b}	3.33 ± 0.55^{ab}	2.64 ± 0.20^{g}	$3.73\pm0.64^{b*}$	$3.52\pm0.41^{e*}$	$3.83\pm0.59^{b*}$	$2.83\pm0.23^{h*}$
6.0	0.5	2.83 ± 0.65^{bc}	3.23 ± 0.40^{e}	3.03 ± 0.56^{b}	2.33 ± 0.35^{h}	$3.27\pm0.58^{c*}$	3.27 ± 0.24^{f}	2.83 ± 0.54^{de}	$2.66\pm0.44^{h*}$

Values followed by different letters within a column are significantly different at 5% level by Duncan's Multiple Range Test. Thirty replicates were used in each treatment. Values marked with * are significantly different at 5% level between 10% coconut milk and 0% coconut milk. Data were recorded after cultured for 60 days on shoot induction and on the sixth subculture

bud multiplication. Coconut milk could promote multiple shoot formation, with a significant difference at 5% level (Table 2).

2.2 Rooting

After the sixth subculture, rooting of shoots was carried out on a full- or half-strength MS medium supplemented with different concentrations of IBA or NAA (Table 3). In the experiment, rooting easily succeeded when the shoots were young (about 2–3 cm measured from base to leaf top). All of these treatments could induce rooting. In most treatments, root initiation appeared when the shoots were transferred to the rooting medium after 10 days. The rooting was affected by the strength of basal medium and the concentration of IBA or NAA. However, rooting percentage was lower on a full strength MS than on a half-strength MS medium at the same concentrations of plant growth regulators. In the absence of any plant growth regulator, only 52.3% and 66.7% of the shoots produced roots in a full- and a half-strength MS medium, respectively. The half-strength MS medium supplemented with 1.0 mg L^{-1} IBA was the best for root induction. All plantlets intended to produce roots after 10 days (Table 3; Plate I: F, G).

Table 3 Effects of various MS salt concentrations and IBA, NAA treatments on rooting of *M. lasiocarpa in vitro* shoots

IBA (mg L^{-1})	NAA (mg L^{-1})	MS			1/2MS		
		Rooting (%)	Number of roots per shoot	Root length (cm)	Rooting (%)	Number of roots per shoot	Root length (cm)
0	0	52.3±11.9^{g}	2.80±0.55^{f}	1.51±0.26^{g}	66.7±9.6^{f*}	3.26±0.87^{g*}	1.62±0.19^{h}
0.2	0	61.7±11.5^{f}	3.53±0.57^{cde}	2.63±0.26^{f}	73.3±9.6^{e*}	4.27±0.58^{de*}	2.82±0.22^{f*}
0.5	0	72.0±9.6^{cd}	4.50±0.63^{b}	3.37±0.32^{e}	84.3±9.4^{c*}	4.80±0.76^{bc}	3.74±0.18^{d*}
1.0	0	92.7±9.8^{a}	5.23±0.63^{a}	4.01±0.37^{a}	100±0.0^{a*}	6.63±1.00^{a*}	4.83±0.32^{a*}
2.0	0	74.7±10.4^{c}	4.33±0.66^{b}	2.90±0.30^{e}	86.0±10.0^{c*}	4.63±0.67^{bcd}	2.82±0.21^{f}
0	0.2	68.3±10.5^{de}	3.80±0.48^{d}	2.95±0.30^{e}	78.3±8.7^{d*}	3.97±0.85^{ef}	2.03±0.24^{g*}
0	0.5	85.7±6.6^{b}	4.60±0.56^{b}	4.04±0.26^{b}	95.0±5.1^{b*}	5.03±0.76^{b*}	4.51±0.33^{b*}
0	1.0	75.7±8.2^{c}	4.16±0.65^{c}	3.60±0.36^{c}	84.0±9.3^{c*}	4.53±0.78^{cd}	3.98±0.38^{c*}
0	2.0	65.7±10.0^{ef}	3.47±0.78^{e}	2.82±0.21^{e}	70.7±12.0^{ef}	3.80±0.71^{f}	3.28±0.27^{e*}

Values followed by different letters within a column are significantly different at 5% level by Duncan's Multiple Range Test. Thirty replicates were used in per treatment. Values marked with * are significantly different at 5% level between MS and 1/2MS. Data were recorded in 4 weeks after transfer of the shoot to rooting medium.

2.3 Transfer *in vitro* propagated plants to soil

The highest transplantation rate (93.5%) was obtained when plantlets were transplanted to planting beds containing a mixture of sand, sieved peat and perliter (1:1:1; v/v) in a greenhouse. After 2 months, the surviving plantlets were transplanted in plastic pots (Plate I: I). Compared to mother plants, the *in vitro* raised plants did not show any phenotypic variation using our safe and effective propagation technique after planting. About 10 000 *M. lasiocarpa* plants were produced successfully in the field and pots (Plate I: H, I).

3 Conclusion and discussion

This protocol described a complete and rapid clonal propagation system for *M. lasiocarpa* using auxiliary bud. In our study, the regeneration frequency (4.10-fold in shoot induction and 4.23-fold in subculture) on MS medium supplemented with 4.0 mg L^{-1} 6-BA, 0.1 mg L^{-1} NAA, 150 mg L^{-1} vitamin C and 10% coconut milk with divided explants. The entire procedure could be completed without callus formation, which could increase possible genetic variability caused by callus formation. The high rates

of multiplication and rooting and successful transfer to a poly bag make this production system useful for *ex situ* conservation and large-scale multiplication of *M. lasiocarpa.*

Activated charcoal and vitamin C could control browning and stimulate shoot growth of *Strelitzia reginae*, *Anemone oronaria*, *Dipterocarpus alatus* and *Dipterocarpus intricatus* [11-12]. Activated charcoal was more effective than vitamin C in reducing browning in *D. alatus* and *D. intricatus* [11]. In our experiment, the use of 150 mg L^{-1} activated charcoal or 150 mg L^{-1} vitamin C or the transplantation of explants on the same fresh medium every 10 days could reduce browning and increase the number of cluster shoots. The effect of vitamin C was better than that of activated charcoal. Addition of activated charcoal in the rooting medium could stimulate [13-14] or inhibit rooting [15]. In our study, activated charcoal improved the potential for adventitious rooting (date not shown).

Coconut milk has been shown to be essential for direct embryogenesis [16] and in enhancement of auxillary shoot growth [17]. Despite studies on chemical composition, nature and level of active growth promoting factors present in the coconut milk remains unknown [18]. Coconut milk contains amino acids, vitamins, sugar and growth substances (cytokinin etc) [16]. In addition, various inorganic ions such as phosphorus, magnesium, potassium and sodium are also reported to be present in coconut milk [18]. However, our results from *M. lasiocarpa* auxiliary shoots initiation showed that coconut milk could increase shoot proliferation rate.

When using undivided or divided explants, higher shoot proliferation rate by divided explants than undivided explants, is the same effect reported in *Curcuma longa* L. [19].

The earlier studies have demonstrated the parameters of the *in vitro* culture conditions are very important for the production of useful plants. The plant growth regulators, nutrient salt strength and the carbon source affected shoot initiation of *Scopolia parviflora* and *Kaempferria galanga* [20]. The multiplication rate and shoot length were significantly higher in cultures supplemented with plant growth regulators in *Saussurea obvallata* (DC.) Edgew [21]. In most of the earlier studies, 6-BA was found to be more effective than kinetin in inducing shoot development [22]. In our experiment, 6-BA was effective, but had inhibitory effect on shoot regeneration and development if the concentration was higher than optimum level (4.0 mg L^{-1}).

Rooting percentage was lower in full strength MS than in half-strength medium at the same concentrations of plant growth regulators, which has been reported for some other medicinal herbs, such as *Saussurea obvallata* (DC.) Edgew [21], *Chlorophytum botivilianum* [23], *Lilium nepalense* D. Don [24].

References

[1] Franchet M A. Un nouveau type de *Musa lasiocarpa* [J]. J Bot, 1889, 3:329–331.

[2] Wu D L, Kress W J. Musaceae [A]. In: Wu C Y, Raven P H. Flora Reipublicae Popularis Sinicae Tomus 24 [M]. Beijing: Science Press, 2001. 314–318.(in Chinese)

[3] Wu C C. Zhi-wu ming-shi tu-kao [M]. Beijing: Reprint. China Book Bureau, 1963. 1844.

[4] Li H W. The Musaceae of Yunnan [J]. Acta Phytotax Sin, 1978, 16 (3):54–64.

[5] Long C L. The application prospective of *Musella lasiocarpa* [A]. In: Collected Papers on the Industriation and Research of Endemic Plants and Animals in Yunnan [C]. Kunming: Yunnan Science and Technology Press, 1997. 172–175.

[6] Wu T L. Flora Reipublicae Popularis Scnicae Tomus 16(2) [M]. Beijing: Science Press, 1981. 3–6.(in Chinese)

[7] Liu A Z, Kress W J, Long C L. The ethnobotany of *Musella lasiocarpa* (Musaceae), an endemic plant of Southwest China [J]. Econ Bot, 2003, 57:279–281.

[8] Guan W L. *In vitro* culture and plantlet regeneration of haustral buds of *Musella lasiocarpa* [J]. Plant Physiol Commun, 2003, 38 (4):358.(in Chinese)

[9] Murashige T, Shoog F. A revised medium for rapid growth and bio-assays with tobacco tissue cultures [J]. Physiol Plant, 1962, 15: 473–497.

[10] Wilkinson L. SYSTAT: The System for Statistics [M]. SYSTAT, Inc. Evanston, IL, 1990. 677.

[11] Linington I M. *In vitro* propagation of *Dipterocarpus intricatus* [J]. Plant Cell Tiss Org Cult, 1991, 27:81–88.

[12] Mensuali-Sodi A, Panizza M, Serra G, et al. Involvement of activated charcoal in the modulation of abiotic and biotic ethylene

levels in tissue-cultures [J]. Sci Hort, 1993, 54:49–57.

[13] Vijaya C D S, Padmaja G. Clonal propagation of mulberry (*Morus indica* L. cultivar M-5) through *in vitro* culture of nodal explants [J]. Sci Hort, 1999, 80:289–298.

[14] Dumas E, Monteuuis O. *In vitro* rooting of micropropagated shoots from Juvenile and mature *Pinus pinaster* explants — influence of activated charcoal [J]. Plant Cell Tiss Org Cult, 1995, 40:231–235.

[15] Lu M C. Micropropagation of *Morus latifolia* plantlet using auxillary buds from mature trees [J]. Sci Hort, 2002, 96:329–341.

[16] Laurain D, Chenieux J C, Tremouillaux-Guiller J. Direct embryogenesis from female haploid protoplasts of *Ginkgo biloba* L., a medicinal woody species [J]. Plant Cell Rep, 1993, 12:656–660.

[17] Boase M R, Wright S, McLeay P L. Coconut milk enhancement of auxillary shoot growth of *in vitro* kiwifruit [J]. New Zealand J Crop Hort Sci, 1993, 21:171–176.

[18] Raghavan V. Diets and culture media for plant embryos [A]. In: Rechcigl M J. CRC Handbook Series in Nutrition and Food [M]. Cleveland, OH: CRC Press, 1977. 361–413.

[19] Prathanturarug S, Soonthornchareonnon N, Chuakul W, et al. High-frequency shoot multiplication in *Curcuma longa* L. using thidiazuron [J]. Plant Cell Rep, 2003, 21:1054–1059.

[20] Shirin F, Kumar S, Mishra Y. *In vitro* plantlet production system for *Kaempferria galanga*, a rare Indian medicinal herb [J]. Plant Cell Tissue Org Cult, 2000, 63:193–197.

[21] Dhar J M. *In vitro* propagation of *Saussurea obvallata* (DC.) Edgew — an endangered ethnoreligious medicinal herb of Himalaya [J]. Plant Cell Rep, 2003, 21:933–939.

[22] Pattnaik S K, Cland P K. Rapid clonal propagation of three mulberries, *Morus cathayana* Hemsl., *M. ihou* Koiz. and *M. serrata* Roxb., through *in vitro* culture of apical shoot buds and nodal explants from mature trees [J]. Plant Cell Rep, 1997, 16:503–508.

[23] Purohit S D, Dave A, Kukda G. Micropropagation of safe musli (*Chlorophytum botivilianum*), a rare Indian medicinal herb [J]. Plant Cell Tiss Org Cult, 1994, 39:93–96.

[24] Wawrosh C, Malla P R, Kopp B. Clonally propagation of *Lilium nepalense* D. Don. A threatened medicinal plant of Nepal [J]. Plant Cell Rep, 2001, 20:285–288.

Explanation of plate

Plate I

Plant regeneration from *in vitro* culture by auxillary bud of *M. lasiocarpa*.

A. Samples of *M. lasiocarpa* grown under greenhouse condition;

B. Inoculated explant from undivided auxillary bud;

C. Inoculated explant from divided auxillary bud;

D. Explants with newly developed shoot;

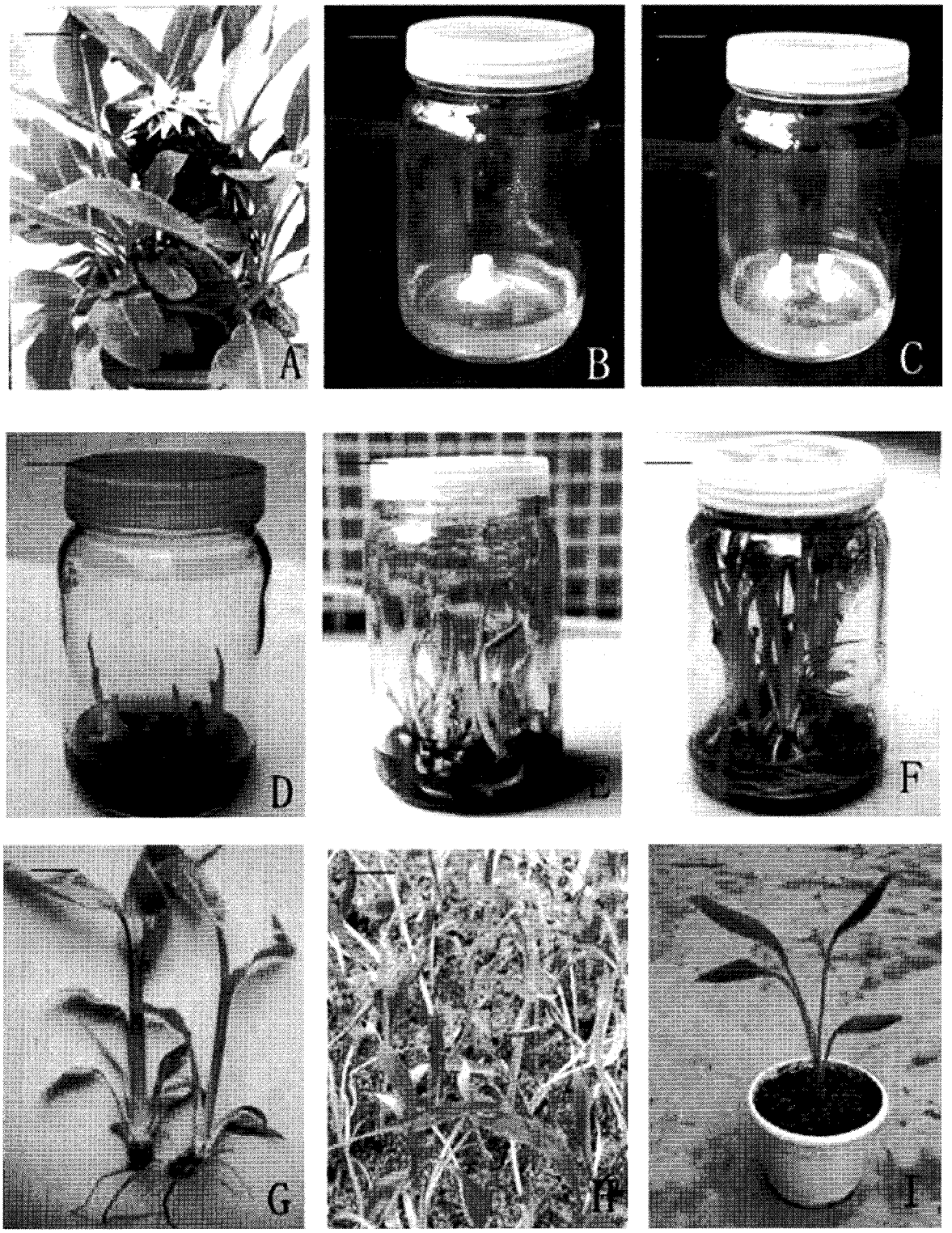
E. Multiplication of *M. lasiocarpa*;

F, G. Rooted plantlets;

H. *M. lasiocarpa* plantlets after acclimatization;

I. Three months after transplant.

Bars: A: 20 cm; B–G: 1.5 cm; H–I: 10 cm.



ZENG Song-jun et al: Plate I