An Efficient Procedure for Regeneration from Leaf-derived Calluses of *Lonicera macranthoides* ‘Jincuilei’, an Important Medicinal Plant

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Abstract. ‘Jincuilei’ is a mutant selected from *Lonicera macranthoides* Hand.-Mazz. It produces abundant flowers that never open with a chlorogenic acid (CGA) content up to 6.0%. Propagation through rooting or grafting has only a 30% survival rate. This study was undertaken to establish an efficient protocol for rapidly regenerating this mutant. Leaf explants were inoculated on Gamborg’s B₅ medium supplemented with different concentrations of 6-benzyladenine (BA) and 2,4-dichlorophenoxyacetic acid (2,4-D). The optimal combination for callus induction was 4.4 μM BA with 2.26 μM 2,4-D, which resulted in 86.7% of leaf explants producing calluses in 4 weeks. Calluses produced from this optimal medium were cultured on B₅ medium containing different concentrations of kinetin (KT) and α-naphthalene acetic acid (NAA). The best formulation for shoot induction was B₅ medium containing 0.9 μM KT and 5.4 μM NAA in which 73.4% of cultured calluses produced shoots in 8 weeks, and shoot numbers ranged from three to six per callus piece (1 cm³). Adventitious shoots were cut and rooted in half-strength Murashige and Skoog medium supplemented with 14.8 μM 3-indolebutyric acid. Roots initiated 10 d after culture, and rooting percentages ranged from 98% to 100%. Plantlets grown in a container substrate in a shaded greenhouse had over a 95% survival rate. During the last 6 years, over four million plantlets were regenerated using this established procedure, and there was no somaclonal variation. Fresh and dry weights of 1000 flowers, CGA contents, and dry flower yields of the regenerated plants were not significantly different from those of the stock ‘Jincuilei’ propagated by cutting, indicating that plants regenerated from this established procedure were stable. This established in vitro culture method has led to rapid commercial production of this medicinal plant on more than 1500 ha of production field.

Lonicera L., commonly known as honeysuckle, is a genus of woody plants and belongs to the family Caprifoliaceae. There are 180 recognized species of deciduous or evergreen, bushy, scandent, twining or creeping shrubs distributed in Asia, Europe, and North America (Huxley, 1994). Flowers are

epigynous, paired, and axillary or in usually six-flowered whorls on terminal spikes with different colors and a pronounced fragrance. Thus, honeysuckles have been widely grown as ornamental plants (Georges et al., 1992; Huxley, 1994; Karhu, 1997). Because of their different growth habitats and great adaptability, a few species such as *L. japonica* Thunb. (Schierenbeck, 2004) and *L. maackii* (Rupr.) Herder. (Gorchov and Trisel, 2003) are also considered invasive species in some regions.

Dried flowers and buds of honeysuckles are called “Jinyinhua” in Chinese or flos lonicerae, which is a famous herb of traditional Chinese medicine. Extracts of flos lonicerae have a wide range of benefits to human health, including anti-inflammation,

bacteriostasis, antiviral, antioxidant, and hematischesis (Lee et al., 1998; Palacios et al., 2002; Peng et al., 2005). Flos lonicerae contains a series of water-soluble phenolic compounds with 3-*O*-caffeoyl-D-quinic acid or chlorogenic acid (CGA) being a major ingredient. In addition to having antipyretic properties, CGA has been reported to suppress *N*-nitrosating reactions and inhibit hepatic glucose 6-phosphatase, which may be a significant factor in the abnormal diabetic state (Arion et al., 1997; Yang et al., 2004). CGA has been studied in *L. confusa* DC., *L. dasystyla* Rehder, *L. hypoglauca* Miq. (Guo and Wan, 2005; Li et al., 2003), *L. japonica* (Lee et al., 1998), and *L. tatarica* L. (Palacios et al., 2002).

Another species, *L. macranthoides* Hand.-Mazz., native to China (Xu and Wang, 1988), was found to contain much higher concentrations of CGA (4.0% to 4.5%) than *L. japonica* (2.2% to 2.5%) (Zhou and Tong, 2003). A survey of genetic resources of *Lonicera* in southwest Hunan province, China, in 1997 to 1998 resulted in the identification of a mutant from a *L. macranthoides* population that was heavily infected with powdery mildew disease (*Microsphaera lonicerae* DC.). The mutant (Fig. 1A), in contrast to its wild type (Fig. 1B), resists the powdery mildew and possesses abundant flowers that never open. Wang et al. (2004) documented that the mutant had an average of 30 nonopened flowers per axillary and the flower longevity ranged from 15 to 25 d compared with 5 to 7 d of the wild-type plants. The mutant was named ‘Jincuilei’. CGA concentration in the nonopened flowers of ‘Jincuilei’ was higher, up to 6.0% compared with 4.3% in its wild-type plants. This mutant, however, does not produce seeds and propagation by cutting or grafting has only a ≈30% survival rate (Wang et al., 2004), which significantly hampers its commercial production as a medicinal plant.

In vitro culture has been shown to be an efficient method of propagating medicinal plants (Debnath et al., 2006; Rout et al., 2000). Research on micropropagation of ‘Jincuilei’ started in 2000. A method of eliminating contaminants from leaf explants was established (Wang et al., 2005). B₅ medium (Gamborg et al., 1968), Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), woody plant medium (Lloyd and McCown, 1980), and White basal medium (White, 1963) were tested for culture of leaf explants. However, a simple and efficient protocol for rapid regeneration of ‘Jincuilei’ was not well developed. The objectives of this study were to establish an efficient procedure for inducing callus and adventitious shoot formation of ‘Jincuilei’ and determine if the regenerated plants maintained the nonopened flower characteristic and high level of CGA.

Materials and Methods

Plant materials. Young leaves of *L. macranthoides* ‘Jincuilei’ were collected from greenhouse stock plants and washed with tap

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Fig. 1. Three-year-old *Lonicera macranthoides* 'Jinculei' regenerated through indirect shoot organogenesis and its wild-type propagated through cuttings. (A) 'Jinculei' with abundant nonopened flowers; (B) the wild-type plants with open flowers and flowers to open.

water followed by a spray of the tissue surface with 75% ethanol for 10 s. The leaves were sterilized in separate bottles containing 0.1% HgCl_2 and two to three drops of Tween 80 for 5 min with occasional agitation (Wang et al., 2005). After pouring out the HgCl_2 solution, leaves were rinsed three times with sterile distilled water and cut into $\approx 0.5\text{-cm}^2$ pieces in sterile petri dishes for inoculation.

Medium preparation. B_5 medium with 0.7% (w/v) agar (ash: 2% to 4%; Mingfu Fujian Agar Co., Ltd., Sanming City, China) and 3.0% (w/v) sucrose was prepared, and the medium pH was adjusted to 5.8 with 1 M KOH before autoclaving at 121°C for 25 min. Plant growth regulator solutions of 6-benzyladenine (BA), kinetin (KT), α -naphthalene acetic acid (NAA), or 2,4-dichlorophenoxyacetic acid (2,4-D) were filter-sterilized and added in different combinations to the autoclaved medium when the temperature dropped to $\approx 50^\circ\text{C}$.

Callus induction. The medium for callus induction was the B_5 medium supplemented with 0, 2.2, 4.4, and $13.3\ \mu\text{M}$ BA with 0, 2.3, 4.5, and $13.6\ \mu\text{M}$ 2,4-D in 16 combinations. The prepared medium was aliquoted into autoclaved 300-mL flasks at 30 mL each. After agar solidification, three leaf explants were placed on the callus induction medium with the adaxial surface up. Based on the percentage of cultured leaf explants in callus formation, the optimal combination of growth regulators for callus induction was identified. A large number of calluses were produced from leaf explants cultured only on this formulation. To minimize the potential of somaclonal variation, only those calluses that were initially induced from leaf explants 4 weeks after culture were used for adventitious shoot induction.

Shoot induction. The calluses were cut into 1-cm^3 pieces (≈ 150 mg in fresh weight) and cultured on shoot induction medium with three callus pieces per flask. The shoot induction medium was B_5 containing KT at 0, 0.5, 0.9, $2.3\ \mu\text{M}$ and NAA at 0, 2.7, 5.4, and $16.1\ \mu\text{M}$ in 16 combinations. Based on the percentage of the cultured callus pieces with shoots, the best formulation for shoot induction was identified. This formulation was used for inducing a large number of shoots from calluses.

Rooting. Adventitious shoots with a height ≈ 2 or 3 cm were excised and rooted in half-strength MS medium containing $14.8\ \mu\text{M}$ 3-indolebutyric acid (IBA). The selection of $14.8\ \mu\text{M}$ IBA was based on a preliminary experiment conducted on half-strength MS medium with different concentrations of IBA. The remaining calluses were discarded because their subculture resulted in quick browning and death.

Culture conditions. Cultures for both callus induction and shoot formation as well as for rooting were maintained in a culture room under a 12-h photoperiod provided by cool-white fluorescent lamps with a photon flux density of $50\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and temperature of $25 \pm 2^\circ\text{C}$.

Transplanting and acclimatization. After washing off the rooting medium with tap water, plantlets were transplanted to plastic pots containing a substrate comprised of 20% clay soil, 40% carbonized rice hull, and 40% coarse sand based on volume. They were grown in a shaded greenhouse under a maximum photosynthetically active photon flux density of $200\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature range of 20 to 28°C , and relative humidity of 70% to 100%. Survival rates of plantlets were recorded 2 months after transplanting.

Comparison of flower yield and chlorogenic acid concentration. Four months after acclimatization, regenerated plants along with cutting propagated stock 'Jinculei' and its wild-type plants were planted on production fields. A nitrogen–phosphate–potash fertilizer with a ratio of 30–10–10 was applied annually at $1500\ \text{kg}\cdot\text{ha}^{-1}$. The experiment was arranged in a completely randomized block design with 10 replications. Two years after planting, all plants started flowering. In the third year, fresh and dry weight of 1000 flowers, dry flower yield of per unit area, and CGA content in the three types of plants were determined.

Chlorogenic acid analysis. CGA was determined by ultraviolet spectrophotometry (Dao and Friedman, 1992). Briefly, fresh flowers from regenerated 'Jinculei', stock 'Jinculei', and wild-type plants were collected and frozen in liquid nitrogen 6 d after the flower appearance (at this time, flowers had not opened in the wild-type plants). The

frozen samples were lyophilized and the dried samples were ground in a Wiley mill to pass a 40-mesh screen. The powders were stored in a refrigerator at -80°C . Samples were extracted using 100% ethyl alcohol for 18 h. The ultraviolet spectrum, 220 to 400 nm, was determined in the ethyl alcohol extract after suitable dilution with ethanol. The concentrations of chlorogenic acid were calculated from the absorption maximum at 326 to 328 nm from a standard curve prepared using chlorogenic acid obtained from Sigma-Aldrich, Co. (St. Louis, MO). The molar extinction coefficient of chlorogenic acid was determined as 19,100 ($n = 10$) compared with a reported Merck Index value of 19,200 (Windholz, 1976).

Data collection and analysis. Each flask was considered an experimental unit for callus induction and shoot formation. There were 10 replications for each treatment. Explants that responded to callus induction were recorded 2 and 4 weeks after culture, and calluses responding to shoot induction were recorded 6 and 8 weeks after inoculation. Data for rooting was collected 15 d after shoots were transferred to rooting medium. After checking normal distribution, analysis of variance for percentages of leaf explants with callus formation and callus pieces with shoot formation were analyzed using SPSS 13.0 for Windows (SPSS, Chicago, IL). Additionally, flower yield and CGA content of regenerated and cutting propagated 'Jinculei' plants as well as the wild-type plants were analyzed. When significant differences ($P < 0.05$) occurred, means were separated using Fisher's protected least significant differences at $P < 0.05$.

Results

Callus induction. The cut edge of leaf explants started to expand 3 d after inoculation, and the entire leaf explants expanded 5 d later (Fig. 2A). Small yellowish particles appeared on explant edges 2 weeks after culture on B_5 medium supplemented with 2.3, 4.5, and $13.6\ \mu\text{M}$ 2,4-D or 2,4-D at these concentrations with 2.2, 4.4, and $13.3\ \mu\text{M}$ BA, respectively (Table 1). Rapid callus formation occurred in 3 to 4 weeks (Fig. 2B). Growth regulator treatments had significant effects on callus formation. No calluses occurred in explants cultured on the B_5 medium devoid of growth regulators or containing BA only. Callus formation frequencies of leaf explants cultured on medium containing 2,4-D only ranged from 10% to 20%, which were significantly lower than those cultured on medium containing both BA and 2,4-D regardless of 2 or 4 weeks of culture. The combinations of BA at the three concentrations with $2.3\ \mu\text{M}$ 2,4-D generally resulted in greater callus formation frequencies than the BA concentrations with the two other 2,4-D concentrations. Although callus formation frequencies on medium containing $2.2\ \mu\text{M}$ BA with $2.3\ \mu\text{M}$ 2,4-D and $4.4\ \mu\text{M}$ BA with $2.3\ \mu\text{M}$ 2,4-D were similar at both 2 and 4 weeks of culture, the combination of $4.4\ \mu\text{M}$

Fig. 2. Morphogenesis of *Lonicera macranthoides* 'Jinculei' through indirect shoot organogenesis. (A) Leaf explants of 'Jinculei' cultured on a callus induction medium 3 d after inoculation. (B) Callus proliferation occurred in leaf explants cultured on B₅ medium containing 4.4 μM 6-benzyladenine and 2.3 μM 2,4-dichlorophenoxyacetic acid 4 weeks after culture. (C) Adventitious shoots initiated from calluses and grew in a shoot induction medium. (D) The vigorous growth of adventitious shoots occurred in B₅ medium containing 0.9 μM kinetin and 5.4 μM α -naphthalene acetic acid. (E) Adventitious shoots rooted in half-strength Murashige and Skoog medium containing 14.8 μM 3-indolebutyric acid. (F) Plantlets were grown in containers with a substrate comprised of 20% clay soil, 40% carbonized rice hull, and 40% coarse sand based on volume in a shaded greenhouse under a maximum photosynthetically active photon flux density of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Bars = 10 mm.

BA and 2.3 μM 2,4-D was selected as the optimal combination for callus induction because it resulted in 86.7% of leaf explants producing calluses 4 weeks after culture.

Shoot induction. Leaf primordia became visible 4 weeks after callus pieces were cultured on shoot induction medium containing both KT and NAA or KT only at concentrations of 0.9 or 2.3 μM ; adventitious shoots appeared 2 weeks later (Fig. 2C). Shoots, however, were not induced from calluses cultured on medium devoid of growth regulators or containing NAA only at the 2.7, 5.4, and 16.1 μM (Table 2). Shoot formation frequencies 6 weeks after callus pieces were cultured on medium containing 0.9 μM KT with 2.7, 5.4, or 16.1 μM NAA were significantly higher (36.7% to 50%) than those cultured on medium containing 0.5 or 2.3 μM KT with 2.7, 5.4, or 16.1 μM NAA (3.3% to

16.7%). The differences became more pronounced 8 weeks after inoculation. Shoot formation frequencies of calluses cultured on medium containing 0.9 μM KT with 2.7, 5.4, or 16.1 μM NAA ranged from 46.7% to 73.4% compared with a range of 10.0% to 26.7% when calluses were cultured on medium containing 0.5 or 2.3 μM KT with 2.7, 5.4, and 16.1 μM NAA, respectively. The best formulation was B₅ medium containing 0.9 μM KT and 5.4 μM NAA in which 73.4% of cultured callus pieces produced adventitious shoots with shoot numbers ranging from three to six (Fig. 2D).

Rooting and acclimatization. Microcuttings were rooted 10 d after culture on rooting medium. On average, four roots were produced per shoot 25 d later (Fig. 2E). Rooting percentage ranged from 98% to 100% (data not shown). Plantlets grown in the container

substrate in the shaded greenhouse had over a 95% survival rate (Fig. 2F). After the establishment of this regeneration protocol, four million plantlets were produced. These regenerated plants were planted on over 1500 ha of production field for commercial 'Jinculei' production.

Comparison of flower yield and chlorogenic acid concentration. No somaclonal variation was observed in the regenerated plants because all the regenerated plants had the same phenotype as 'Jinculei' propagated by cutting. Regenerated plants produced abundant nonopened flowers. Fresh and dry weight of 1000 flowers, CGA content, and dry flower yield of the regenerated 'Jinculei' were similar to those of 'Jinculei' plants propagated by cutting (Table 3). These results indicated that plants regenerated from this established method had no variation in flower yield and CGA content from 'Jinculei' propagated by cutting. However, such measured parameters varied significantly between 'Jinculei' and its wild-type plants.

Discussion

In vitro culture has been used to manipulate several *Lonicera* species, and in some instances, whole plants have been regenerated. Shoot proliferation was achieved using single nodes of *L. periclymenum* L. (Boonnour et al., 1988), *L. caerulea* L. (Karhu, 1997), and *L. tatarica* (Palacios et al., 2002). Indirect shoot organogenesis was established using leaf explants of *L. japonica* (Georges et al., 1993) and *L. nitida* E. H. Wilson (Cambeceades et al., 1991) as well as leaf protoplasts of *L. nitida* (Ochatt, 1991). However, there has been limited information available regarding the regeneration of *L. macranthoides*. In this study, we established an efficient procedure for regeneration of a mutant of *L. macranthoides* through indirect shoot organogenesis, i.e., callus induction from leaf explants on B₅ medium containing 4.4 μM BA and 2.3 μM 2,4-D in 4 weeks; shoot induction from calluses on B₅ medium supplemented with 0.9 μM KT with 5.4 μM NAA in 8 weeks; and rooting of adventitious shoots in half-strength MS medium containing 14.8 μM IBA in 4 weeks.

The callus induction method is simple and convenient with the use of leaf explants. Compared with the other callus induction media for other *Lonicera*, this established medium is more effective because 86.7% of leaf explants of 'Jinculei' produced calluses in only 2 to 4 weeks after culture. A callus induction medium, MS containing 2.3 μM N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (TDZ) and 2.9 μM 3-indoleacetic acid developed by Cambeceades et al. (1991), induced 56.6% of leaf explants producing calluses in 15 weeks. Callus induction from leaf explants of *L. japonica* on Linsmaier and Skoog (1965) medium supplemented with 10.7 μM NAA and 2.7 μM BA took 25 weeks (Georges et al., 1993). Additionally, Linsmaier and Skoog (1965) medium containing 0.45 μM

Table 1. Frequency of callus formation from leaf explants of *Lonicera macranthoides* 'Jinculei' cultured 2 and 4 weeks on B₅ medium^a supplemented with different concentrations of BA and 2,4-D.^y

Growth regulators (μM)		Frequency of explants with callus formed (%)	
BA	2,4-D	2 weeks	4 weeks
0.0	0.0	0.0 ± 0.00 f ^x	0.0 ± 0.00 f
0.0	2.3	10.0 ± 0.16 e	23.3 ± 0.23 e
0.0	4.5	20.0 ± 0.23 d	36.7 ± 0.25 d
0.0	13.6	16.7 ± 0.24 de	36.7 ± 0.19 d
2.2	0.0	0.0 ± 0.00 f	0.0 ± 0.00 g
2.2	2.3	43.3 ± 0.23 a	83.4 ± 0.18 a
2.2	4.5	36.7 ± 0.25 b	66.7 ± 0.22 b
2.2	13.6	26.7 ± 0.26 c	53.3 ± 0.17 c
4.4	0.0	0.0 ± 0.00 f	0.0 ± 0.00 g
4.4	2.3	43.3 ± 0.23 a	86.7 ± 0.17 a
4.4	4.5	40.0 ± 0.21 a	73.7 ± 0.23 ab
4.4	13.6	33.3 ± 0.22 b	60.0 ± 0.21 b
13.3	0.0	0.0 ± 0.00 f	0.0 ± 0.00 f
13.3	2.3	40.0 ± 0.21 a	66.7 ± 0.25 b
13.3	4.5	33.0 ± 0.22 b	56.7 ± 0.23 bc
13.3	13.6	26.7 ± 0.21 c	46.7 ± 0.17 c

^aGamborg B₅ medium supplemented with 0.7% (w/v) agar and 3.0% (w/v) sucrose.

^yThree leaf explants per 300-mL flask and 10 flasks per treatment.

^xDifferent letters within a column represent significant difference at $P < 0.5$ by Fisher's protected least significant difference test.

BA = 6-benzyladenine; 2,4-D = 2,4-dichlorophenoxyacetic acid.

Table 2. The frequency of shoot formation from cultured callus pieces of *Lonicera macranthoides* 'Jinculei' 6 and 8 weeks after inoculation on B₅ medium^a supplemented with different concentrations of KT and NAA.^y

Growth regulators (μM)		Frequency of callus pieces with shoot formation (%)	
Kinetin	NAA	6 weeks	8 weeks
0.0	0.0	0.0 ± 0.00 f ^x	0.0 ± 0.00 g
0.0	2.7	0.0 ± 0.00 f	0.0 ± 0.00 g
0.0	5.4	0.0 ± 0.00 f	0.0 ± 0.00 g
0.0	16.1	0.0 ± 0.00 f	0.0 ± 0.00 g
0.5	0.0	0.0 ± 0.00 f	6.7 ± 0.14 f
0.5	2.7	6.7 ± 0.14 d	13.3 ± 0.17 e
0.5	5.4	10.0 ± 0.16 cd	16.7 ± 0.24 e
0.5	16.1	6.7 ± 0.14 d	13.3 ± 0.23 e
0.9	0.0	13.3 ± 0.17 c	26.7 ± 0.21 d
0.9	2.7	36.7 ± 0.25 b	56.7 ± 0.23 b
0.9	5.4	50.0 ± 0.24 a	73.4 ± 0.21 a
0.9	16.1	30.0 ± 0.25 b	46.7 ± 0.23 c
2.3	0.0	6.7 ± 0.14 d	16.7 ± 0.24 e
2.3	2.7	6.7 ± 0.14 d	13.3 ± 0.23 e
2.3	5.4	16.7 ± 0.24 c	26.7 ± 0.26 d
2.3	16.1	3.3 ± 0.11 e	10.0 ± 0.23 e

^aGamborg B₅ medium supplemented with 0.7% (w/v) agar and 3.0% (w/v) sucrose.

^yThree callus pieces (1 cm³) per 300-mL flask and 10 flasks per treatment.

^xDifferent letters within a column represent significant difference at $P < 0.5$ by Fisher's protected least significant difference test.

KT = kinetin or 6-furfurylaminopurine; NAA = α -naphthalene acetic acid.

Table 3. Fresh and dry weight of 1000 flowers, flower chlorogenic acid (CGA) content, and dry flower yield of the wild-type *Lonicera macranthoides*, stock 'Jinculei' propagated by cuttings and regenerated 'Jinculei' in their third year after planting on the production field.

Plant	Flower fresh wt (g/1000 flowers)	Flower dry wt (g/1000 flowers)	CGA content (%)	Dry flower yield (kg·ha ⁻¹)
Wild type	56.4 b ^z	13.3 b	4.3 b	1,462.8 b
Stock Jinculei	73.4 a	17.6 a	5.9 a	3,377.2 a
Regenerated Jinculei	74.1 a	17.9 a	6.0 a	3,385.6 a

^zMeans followed by different letters are significantly different at $P < 0.05$ by Fisher's protected least significant difference test. Data represent means of 10 replications.

2,4-D resulted in the rapid necrosis of leaf explants of *L. japonica* (Georges et al., 1993). There was no necrosis on leaf explants of *L. macranthoides* 'Jinculei' cultured on B₅ medium containing up to 13.6 μM 2,4-D. The high efficiency of callus induction in *L. macranthoides* could be attributed to the genetic makeup of this species, the B₅

medium, appropriate growth regulators, or a combination of the three factors.

The shoot induction medium for 'Jinculei' was either equal to or more effective than those reported with the other *Lonicera* species. The occurrence of shoots from callused explants was 74% in *L. nitida* after 8 weeks of culture on half-strength MS medium containing 2.3

μM TDZ and 20 μM 2,3,5-triiodobenzoic acid (Cambecedes et al., 1991) and 44% for calluses of *L. japonica* cultured on woody plant medium containing 4.4 to 44.4 μM BA for 13 weeks (Georges et al., 1993). Adventitious shoots of *L. macranthoides* 'Jinculei' appeared within 6 weeks after callus culture, and up to 73.4% of cultured callus pieces produced shoots after 8 weeks on B₅ medium containing 0.9 μM KT with 5.4 μM NAA. BA is considered more effective in shoot induction than KT (Georges et al., 1993). In one of our preliminary shoot induction studies, BA at concentrations of 4.4, 13.3, and 22.2 μM only or with NAA at 2.7, 5.4, and 16.1 μM, respectively, did not result in shoot formation frequencies greater than 50% (data not shown). On other hand, KT with NAA was found to be more effective in shoot induction of 'Jinculei'.

In general, the most difficult stage for regeneration of woody species is the induction of roots from shoots (Bonga, 1977). Adventitious shoots of 'Jinculei' readily rooted in half-strength MS medium containing 14.76 μM IBA with a rooting percentage as high as 100% (data not shown). This ease in rooting characteristic was similar to that of *L. nitida* (Cambecedes et al., 1991; Ochatt, 1991) in which high endogenous IBA in *Lonicera* was assumed to be a factor.

Indirect shoot organogenesis is often associated with somaclonal variation (Chen and Henny, 2006; Skirvin et al., 1994). The duration of in vitro culture has been shown to be an important factor. Somaclonal variation generally increases with the time that a culture has been maintained in vitro (Bouman and De Klerk, 1997; Skirvin et al., 1994). No somaclonal variation was observed among four million regenerated plants because all the regenerated plants had the same morphology as 'Jinculei' propagated by cutting. The flower yield and CGA content of the regenerated plants were comparable to those of cutting-propagated 'Jinculei'. In addition to the plant genotype, the high genetic fidelity among the regenerated plants could be in part attributed to this established regeneration procedure in which calluses used for shoot induction were directly induced from leaf explants and not from subculture. Additionally, the regeneration process from culture of leaf explants to excision of adventitious shoots took only ≈12 weeks, which might also help minimize the potential for somaclonal variation. Nevertheless, this established method resulted in the planting of over 1500 ha of regenerated 'Jinculei'. A large quantity of nonopened flowers has been produced and processed as flos *Lonicerae* for medicinal use.

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