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An efficient adventitious shoot regeneration system for ramie (*Boehmeria nivea* Gaud) using thidiazuron

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ABSTRACT. An efficient culture system for direct adventitious shoot regeneration of plantlet from cotyledon of ramie (*Boehmeria nivea* Gaud) was established and factors affecting shoot regeneration efficiencies were evaluated. Cotyledons excised from 4-day-old seedlings showed the highest regeneration capacity. Various concentrations of thidiazuron (TDZ) and indoleacetic acid (IAA) gave rise to adventitious shoots with different efficiencies. The cultures of cotyledons from 4-day-old ramie seedlings on MS medium supplemented with 2.27 μM TDZ and 0.057 μM IAA were shown to have the highest efficiency (83.6%) of shoot regeneration among the four auxin supplements (*2,*4-D; IAA; IBA; NAA). Regeneration of shoot in six cultivars was genotype-dependent, '5041-3' being the most responsive one. The regenerated shoots were transferred to hormone-free medium for shoot elongation, after successfully rooted on half-strength MS medium supplemented with 0.27 μM NAA. The rooted plantlets with 4-5 leaves were transplanted to greenhouse for further growth. To our knowledge, this is the first report on efficient regeneration of plantlet from cotyledon of ramie, which will be of value for genetic improvement in the near future.

Keywords: *Boehmeria nivea* Gaud; Cotyledon; Organogenesis; Ramie; Thidiazuron.

Abbreviations: BA, Benzyladenine; **FCM,** Flow cytometry; **2, 4-D,** 2, 4-Dichlorophenoxyacetic acid; **IAA,** Indoleacetic acid; **IBA,** Indolebutyric acid; **MS medium,** MS medium basal medium; **NAA,** α-Naphthaleneacetic acid; **TDZ,** thidiazuron (*N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea).

INTRODUCTION

Ramie (*Boehmeria nivea* Gaud), commonly known as China grass, is a perennial fiber plant in Urticaceae, which has been grown in China for thousands of years and was used for Chinese burial shrouds over 2,000 years. It is used for the production of textiles and ropes because it is extremely absorbent, dries quickly, dyes fairly easy, resists shrinkage, and is unusually tolerant with bacteria, mildew and insect attacks. Ramie is frequently used in fabric blends due to its poor elasticity and brittleness. Consequently, when added to cotton, ramie can increases strength, color and luster without compromising the flexibility of the fabric.

Improvement of ramie mainly focused on fiber quality, yield and resistance. However, traditional breeding of ramie is limited owing to its complex genetic background. Comparatively, *in vitro* techniques can provide alternative means for cultivar improvement via *Agrobacterium*-mediated transformation. Successful application of *in vitro* methods is largely dependent on a reliable regeneration system, which, nevertheless, has not been established in ramie. Since the first publication on tissue culture of ramie was reported over two decades ago (Zhou et al., 1980), efforts have been made to regenerate ramie plantlets from different explants such as cotyledons (without regeneration data) (Huang et al., 1980), stem segments (Pan et al., 1995), hypocotyls (Huang et al., 1981) and leaves (Guo et al., 1998; Pan et al., 1995). It is noted that in all of the above studies plants were regenerated via morphogenesis from callus induced from the explants. The callus was always subcultured for several cycles before plant regeneration. One disadvantages of such process is the recalcitrance of the callus to differentiation (Pan et al., 1995), which negatively affected the regeneration efficiency. Furthermore, a low rate of regeneration (not more than 50%) in these studies limited the application of previous protocols in genetic transformation, which, so far, has been performed in work (Dusi et al., 1993). Therefore, it is necessary to establish a reliable and efficient regeneration system of ramie, which can be employed for genetic transformation in the future.

Cotyledon, which has been widely used for many other plants (Colijin-Hooymans et al., 1994; Yang et al., 2001; Han et al., 2004; Sul III-Whan et al., 2004; Zhang et al., 2005), was scarcely used in ramie tissue culture. Thidiazuron (TDZ), a substitute urea with both cytokinin activ-

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ity and auxin activity (Mok et al., 1982; Visser et al., 1992; Murthy et al., 1998), has been widely used in plant tissue culture as powerful cytokinin (Huettman et al., 1993; Lu et al., 1993; Colijin-Hooymans et al., 1994; Ahroni et al., 1997; Kim et al., 1997; Gless et al., 1998; Li et al., 2000; Gu et al., 2005). But it has never been reported in ramie tissue culture. Therefore, in the present study, attempts were made to establish an efficient regeneration system based on culture of ramie cotyledon explants and use of TDZ as an important hormone, which is expected to hold potential for genetic transformation in the future.

MATERIAL AND METHODS

Plant material

Mature seeds of six cultivars of ramie, 'Luzhuqing', '5041-3', '1690', 'Dama No. 1', 'Nianhong' and 'Huangpigun', were collected from Ramie Germplasm Resources Garden located at Huazhong Agricultural University, China. The seeds were first soaked in 70% (v/v) ethanol for 30 seconds and then treated with 10% (w/v) sodium hypochlorite (NaOCl) for 15 min, followed by 3-4 rinses with sterile water for surface sterilization. The sterilized seeds were germinated on half-strength MS medium (Murashige and Skoog, 1962) without growth regulators to produce cotyledons. When cotyledons were observed and fully developed, they were carefully excised from each seedling for culture without any other wounding.

Culture of cotyledons

The cotyledons from the cultivar 'Luzhuqing' were collected at 2-day intervals from the seedlings 2 to 12 days after germination, which were cultured on MS medium supplemented with 2.27 μM TDZ and 0.057 μM IAA. Each cotyledon was placed with its abaxial side contacting the medium. Since 4-day-old seedlings were proven to be the optimum donor of cotyledons in the experiment described above, they were employed to study effect of various plant hormone combinations on shoot regeneration. Cotyledons excised from 4-day-old seedlings were cultured on MS medium supplemented with 2.27 μM TDZ and either of IAA (0.0285, 0.057, 0.285, 1.43 or 2.85 μM), IBA (0.0246, 0.049, 0.246, 1.23 or 2.46 μM), NAA (0.0268, 0.054, 0.268, 1.34 or 2.68 μM) or 2,4-D (0.0226, 0.045, 0.226, 1.13 or 2.26 μM). The effects of different concentrations of TDZ and IAA on shoot regeneration were further tested using cotyledons excised from 4-day-old seedlings that were cultured on MS medium supplemented with 0.045, 0.23, 1.14, 2.27 or 4.54 μM TDZ in combination with 0, 0.028, 0.057, 0.285 or 2.85 μM IAA, respectively. Finally, in order to test the selected regeneration systems for different genotypes, cotyledons excised from 4-day-old seedlings of six ramie cultivars, '5041-3', 'Luzhuqing', '1690', 'Dama No. 1', 'Nianhong' and 'Huangpigun', were cultured on MS medium supplemented with 2.27 μM TDZ and 0.057 μM IAA or with 2.27 μM TDZ and 0.054 μM NAA.

Shoot elongation and rooting

After culture for 8 weeks, the adventitious shoots regenerated from cotyledon explants were transferred to hormone-free MS medium for shoot elongation. When the shoots reached 2 cm in height, they were transferred onto half-strength MS medium supplemented with 0.27 μM NAA (Guo et al., 1998) for rooting. Young plantlets with 4-5 leaves were transplanted to plastic pots, in which soil, vermiculite and sand (2:1:1) were blended, before they were moved to the greenhouse for further growth.

Culture conditions

All the experiments except for plantlets acclimation were conducted in 150 ml conical flask containing 30 ml medium. All of the media were supplemented with 30 g/l glucose, solidified with 8 g/l agar, with pH adjusted to 5.8 prior to autoclaving at 121°C for 20 min. Cultures were incubated at 25±2°C under a 16/8-h (light/dark) photoperiod with a light intensity of 3,000 lux.

Data collection and statistical analysis

All experiments were conducted 3 replicates with 3 150 ml conical flasks in each and 12-15 explants were cultured in every 150 ml conical flask. Data on the percentage of shoot formation and average number of shoots per explant were analyzed using SAS ver 6.12 (SAS Institute, 1995, Cary, N.C.). Analysis of variance (ANOVA) was used to test the statistical significance, and the significance of differences among means was carried out using Duncan's (1955) multiple range test at P=0.05.

Ploidy analysis by flow cytometry (FCM)

The ploidy level of the regenerated plantlets was determined with flow cytometry as described by Xu et al. (2006). 40 plantlets were selected randomly from the regenerated plantlets described above and 1.5 cm^2 young leave was cut into small pieces in nuclear extraction buffer (solution A of High Resolution Kit for Plant DNA, Partec, Germany) respectively. Then it was filtered through a nylon sieve (Partec CellTrics, Germany) with mesh diameter of 30 μm, followed by adding a staining solution containing DAPI (solution B of the kit). The samples were then measured by a flow cytometer (PA-I, Partec). The relative DNA content of 'Luzhuqing' seedlings was used as a ploidy standard. The ploidy of the cell lines was calculated by comparing the relative fluorescence intensity.

RESULTS AND DISCUSSION

Effect of donor plant age on shoot induction

Effect of cotyledons sampled at different time after germination was evaluated, which showed that cotyledon age influenced the efficiency of adventitious shoot formation (Table 1). Cotyledons excised from 4-day-old seedlings gave rise to the highest percentage of shoot formation (51.1%), whereas 12-day-old cotyledons gave rise to the

Table 1. Effect of cotyledon explant age of 'Luzhuqing' cultivar on percentage of shoot formation (SF%) and average number of shoots per explant (AS) (means ± SE) 8 weeks after culture.

Cotyledon age	SF%[a]	AS[a]
2 d	37.8 ± 7.3 b	5.6 ± 1.1 a
4 d	51.1 ± 7.8 a	5.5 ± 0.4 ab
6 d	28.1 ± 4.5 c	5.1 ± 1.2 ab
8 d	18.3 ± 4.8 d	4.7 ± 1.0 ab
10 d	13.4 ± 3.0 de	4.6 ± 0.9 ab
12 d	7.9 ± 2.5 e	3.6 ± 1.7 b

[a]Different letters in the same column shows that the values are significantly different at P<0.05.

lowest percentage (7.9%) and the least number of shoots per explant (3.6). Regeneration rate was inversely related to the age of plants within 4 days after culture. In addition, 2-day-old cotyledons produced the largest number of shoots per explant (5.6), but there was no significant difference between the 2-day-old and 4-day-old seedlings. These results seemed to reveal that the regeneration potential of cotyledon explants depended on the developmental stage of the donor plant, in agreement with that reported in cucumber by Colijn-Hooymans et al. (1994) and in bottle gourd by Han et al. (2004). However, it did not agree with Pan et al. (1995) who discovered that regeneration rate increased with the age of hypocotyls sampled from 7 to 16-day-old of ramie seedlings, which might be due to the different explant types and/or genotypes.

Effect of different auxins on shoot induction

Different auxins in combination with TDZ have been used for plant regeneration in other plant species, i.e. gypsoghila (Ahroni et al., 1997), sweetgum (Kim et al., 1997), winter jujube (Gu et al., 2005). Herein, we also investigated effects of auxin on shoot induction, which demonstrated that culture of cotyledons on MS medium added with 2.27 μM TDZ and different auxins led to significant differences in percentage of shoot formation and average number of shoots per explant (Table 2). The optimum concentration of a given auxin to induce plant regeneration from cotyledons of ramie was different. IAA at 0.057 μM and NAA at 0.054 μM were optimal for shoot regeneration compared with IBA at 1.23 μM and 2,4-D at 1.13 μM for regeneration rate and number of shoots per explant. The regeneration rate and number of shoots per explant were reversely related to IAA concentration over 0.057 μM and NAA concentration over 0.054 μM, whereas they increased with IBA and 2,4-D except at the highest concentration. In addition, IAA at concentrations higher than 0.057 μM, NAA at concentrations higher than 0.054 μM, IBA higher than 1.23 μM and 2,4-D higher than 1.13 μM, combined with TDZ, induced more callus

Table 2. Effect of different auxins on percentage of shoot formation (SF%) and average number of shoots per explants (AS) from cotyledons of 'Luzhuqing' (means ± SE) 8 weeks after culture

Auxins (μM)				SF%[a]				AS[a]			
IAA	NAA	IBA	2,4-D	IAA	NAA	IBA	2,4-D	IAA	NAA	IBA	2,4-D
0.0285	0.0268	0.0246	0.0226	26.8 ± 1.8 b	9.1 ± 0.8 c	22.9 ± 1.9 b	6.1 ± 0.2 b	2.5 ± 0.2 c	4.0 ± 1.0 b	2.5 ± 0.5 b	1.3 ± 0.6 c
0.057	0.054	0.049	0.045	54.3 ± 4.2 a	47.8 ± 3.8 a	25.0 ± 5.5 ab	21.3 ± 3.3 a	4.6 ± 0.4 a	5.2 ± 0.8 a	2.6 ± 0.1 b	2.1 ± 0.4 bc
0.285	0.268	0.246	0.226	25.0 ± 3.6 b	21.2 ± 5.4 b	28.0 ± 7.0 ab	22.8 ± 2.6 a	3.9 ± 0.8 ab	4.4 ± 0.4 ab	2.8 ± 0.2 b	3.2 ± 0.5 a
1.43	1.34	1.23	1.13	24.9 ± 1.8 b	16.0 ± 3.5 b	32.8 ± 3.2 a	26.0 ± 5.7 a	3.6 ± 0.5 b	3.9 ± 0.4 b	3.7 ± 0.5 a	3.5 ± 0.7 a
2.85	2.68	2.46	2.26	22.1 ± 2.6 b	5.9 ± 0.3 c	28.8 ± 4.7 ab	20.4 ± 1.2 a	2.6 ± 0.4 c	3.6 ± 0.2 b	3.2 ± 0.5 ab	3.0 ± 0.3 ab

[a]Different letters in the same column shows that the values are significantly different at P<0.05.

from cotyledon explants than those at lower concentration auxin (data not shown). Our results indicated that low concentration of IAA combined with TDZ was the best condition for inducing shoots from ramie cotyledon.

Effect of concentrations of TDZ and IAA on shoot induction

In order to test the effect of TDZ and IAA on shoot induction, five concentrations of TDZ in combination with five concentrations of IAA were used, which showed percentage of shoot formation of two ramie cultivars ('Luzhuqing' and '5041-3') increased with increase in TDZ except the highest concentration at the same IAA levels (Table 3). The highest percentage of shoot formation (54.3% and 86.2%, respectively) and the largest number of shoots per explant (4.6 and 4.4, respectively) for both genotypes were obtained when 2.27 μM TDZ and 0.057 μM IAA were used. ANOVA showed significant differences in both percentage of shoot formation and average number of shoots per explant of 'Luzhuqing'

Table 3. Effect of TDZ and IAA concentration on percentage of shoot formation (SF%) and average number of shoots per explants (AS) from cotyledons of 'Luzhuqing' and '5041-3' (means ± SE) 8 weeks after culture.

TDZ (μM)	IAA (μM)	SF%[a]		AS[a]	
		Luzhuqing	5041-3	Luzhuqing	5041-3
0.045	0	0.0 ± 0.0 i	0.0 ± 0.0 o	0.0 ± 0.0 i	0.0 ± 0.0 j
	0.029	14.8 ± 1.7 g	7.7 ± 0.6 lmn	1.3 ± 0.3 h	1.7 ± 0.6 hi
	0.057	6.5 ± 0.8 h	9.9 ± 3.8 klmn	1.7 ± 0.3 fgh	2.7 ± 0.4 cdefg
	0.285	8.5 ± 1.4 h	21.5 ± 1.5 feg	2.0 ± 0.3 efg	2.0 ± 0.3 ghi
	2.85	0.0 ± 0.0 i	12.5 ± 0.8 ijkl	0.0 ± 0.0 i	1.7 ± 0.3 ghi
0.23	0	0.0 ± 0.0 i	4.9 ± 4.3 no	0.0 ± 0.0 i	1.3 ± 1.2 hi
	0.029	15.8 ± 1.7 fg	16.2 ± 0.7 ghij	1.4 ± 0.3 h	1.9 ± 0.6 ghi
	0.057	16.9 ± 5.4 efg	17.5 ± 0.8 fghi	3.0 ± 0.4 c	2.8 ± 0.6 cdef
	0.285	17.4 ± 2.4 efg	24.3 ± 5.7 de	2.2 ± 0.3 def	2.1 ± 0.2 fghi
	2.85	16.7 ± 4.1 efg	14.3 ± 1.0 hijk	1.6 ± 0.1 gh	2.0 ± 0.5 ghi
1.14	0	7.9 ± 1.9 h	6.8 ± 0.3 mn	1.7 ± 0.3 fgh	1.7 ± 0.6 hi
	0.029	19.4 ± 2.4 cdefg	18.3 ± 4.3 fgh	2.6 ± 0.5 cd	2.3 ± 0.3 efgh
	0.057	23.5 ± 4.7 bcd	32.6 ± 2.9 c	3.7 ± 0.3 b	3.0 ± 0.2 bcde
	0.285	18.3 ± 1.7 defg	28.7 ± 2.1 cd	2.5 ± 0.5 cde	2.7 ± 0.4 cdefg
	2.85	21.4 ± 8.1 bcdef	17.8 ± 3.8 fghi	1.9 ± 0.1 fg	2.5 ± 0.2 cdefg
2.27	0	15.7 ± 1.2 fg	22.7 ± 3.5 ef	2.2 ± 0.4 def	3.3 ± 0.5 bc
	0.029	26.7 ± 1.8 b	48.3 ± 6.5 b	2.5 ± 0.2 cde	3.1 ± 0.3 bcd
	0.057	54.3 ± 4.2 a	86.2 ± 3.4 a	4.6 ± 0.4 a	4.4 ± 0.5 a
	0.285	24.9 ± 1.8 bc	43.9 ± 5.4 b	3.9 ± 0.8 b	3.7 ± 0.6 ab
	2.85	22.1 ± 2.6 bcde	29.8 ± 3.4 c	2.6 ± 0.4 cd	2.6 ± 0.2 cdefg
4.54	0	7.8 ± 2.7 h	13.7 ± 0.5 hijk	1.3 ± 0.3 f	1.7 ± 0.3 hi
	0.029	16.3 ± 2.7 fg	18.6 ± 3.8 fgh	1.5± 0.1 gh	2.0 ± 0.3 ghi
	0.057	26.5 ± 9.2 b	44.3 ± 5.2 b	2.5± 0.2 cd	2.3 ± 0.3 defgh
	0.285	23.2 ± 1.8 bcd	18.8 ± 1.2 fgh	2.2 ± 0.3 def	2.5 ± 0.5 cdefg
	2.85	20.8 ± 2.5 cdef	12.1 ± 1.1 jklm	2.0 ± 0.0 efg	2.1 ± 0.4 fghi
Analysis of variance		SF%		AS	
Source of variation		F-test[b]		F-test	
TDZ		86.55**	267.54**	99.80**	30.77**
IAA		86.29**	161.67**	96.11**	15.03**
TDZ×IAA		10.17**	29.27**	5.55**	1.58

[a]Different letters in the same column shows that the values are significantly different at $P<0.05$.

[b]F-test: *, ** Significant at $P\leq0.05$ or 0.01, respectively.

among different concentrations of TDZ and IAA, and significant interactions existed between TDZ and IAA. Similar results were obtained in '5041-3' except that no significant interaction between TDZ and IAA was found in average number of shoots per explant. The results showed that TDZ, which has been proven to be powerful in stimulating shoot regeneration in many plant species including gypsophila (Ahroni et al., 1997), oat (Gless et al., 1998), cucumber (Colijin-Hooymans et al., 1994), Huang-qin (Li et al., 2000), sweetgum (Kim et al., 1997) and winter jujube (Gu et al., 2005), was an effective plant growth regulator for the induction of shoot regeneration from ramie cotyledons. However, in this study, it is worth noting that percentage of shoot formation was inhibited when 4.54 μM TDZ was used compared with TDZ at lower concentration. In addition, the shoot elongation in this study was also retarded by 4.54 μM TDZ, since the regenerated shoots induced on medium supplemented with 4.54 μM TDZ were shorter than those at lower concentration (below 1.14 μM) of TDZ within 8 weeks (data not shown). The inhibition of TDZ observed herein was possibly due to toxicity of TDZ, as has been reported elsewhere (Huettman et al., 1993; Lu et al., 1993; Kim et al., 1997).

Genotypic response to the shoot regeneration system

Cotyledons of six genotypes were cultured on MS medium containing 2.27 μM TDZ and 0.057 μM IAA or 2.27 μM TDZ and 0.054 μM NAA to investigate the genotypic response to *in vitro* culture (Table 4). Regeneration of shoots occurred in all of the genotypes with regeneration rate higher than 50% in most cases. Regeneration rate of '5041-3' was the highest, whereas the least responsive genotype was 'Huangpigun'. ANOVA analysis showed significant differences in percentage of shoot formation among the six cultivars, which indicated that shoot regeneration was genotype-dependent, in agreement with the findings in ramie (Pan et al., 1995), carnation (Henni Kallak et al., 1997), melon (Ficcadenti et al., 1995), rape (Khehra et al., 1992) and red pepper (Christopher et al., 1996).

Plant regeneration, elongation, rooting and transplantation

Cotyledons cultured on MS medium supplemented with TDZ and auxins began to swell 7 day later, and the first bud formation was observed at the cutting edge 3 weeks after culture (Figure 1A, B). Some cotyledons only produced one shoot (Figure 1C), but others produced multiple shoots (Figure 1D). It is noted that shoot regenerated from the cutting edge of cotyledon explant without callus phase. It is known that regeneration from callus that has been maintained for several cycles is always coupled with somaclonal variation (Cassells and Curry, 2001). Therefore, regeneration from cotyledon directly may minimize somaclonal variation (Skirvin et al., 1994; Cassells and Curry, 2001; Šušek et al., 2002), which seems superior to the previous reports on ramie tissue culture involving callus (Huang et al., 1980; Huang et al., 1981; Pan et al., 1995; Guo et al., 1998).

The regenerated shoots were cut carefully from cotyledon explants 8 weeks after culture and were cultured on hormone-free MS medium for shoot elongation. The shoots grew quickly on MS medium and reached 2 cm in height within one month (Figure 1E), which were then transferred to half-strength MS medium added with 0.27 μM NAA to induce root. About 95% of the shoots rooted successfully (Figure 1F). The rooted plantlets were transplanted to plastic pots containing soil, vermiculite and sand (2:1:1) and almost all of the plantlets survived in the greenhouse (Figure 2A, B).

Ploidy analysis of regenerated plantlets

FCM, a technique which has been described as a timesaving method for ploidy analysis (Xu et al., 2006), was used to analyze the ploidy levels of 40 plantlets

Table 4. Effect of genotype on percentage of shoot formation (SF%) and average number of shoots per explants (AS) (means ± SE) 8 weeks after culture using cotyledons explants from 4-day-old seedlings.

Genotypes	SF%[a]		AS[a]	
	TDZ/IAA[b]	TDZ/NAA[c]	TDZ/IAA[b]	TDZ/NAA[c]
5041-3	86.2 ± 3.4 a	64.5 ± 5.2 a	4.4 ± 0.6 a	5.2 ± 0.6 ab
1690	52.9 ± 5.4 cd	56.2 ± 9.9 ab	4.3 ± 0.4 a	5.5 ± 0.5 a
Luzhuqing	62.2 ± 3.9 b	47.8 ± 3.8 b	4.6 ± 0.4 a	4.8 ± 0.6 ab
Dama No. 1	59.5 ± 3.5 bc	48.2 ± 1.8 b	4.5 ± 0.4 a	5.2 ± 0.5 ab
Nianhong	55.0 ± 2.9 c	50.1 ±11.4 b	4.4 ± 0.4 a	4.6 ± 0.5 bc
Huangpigun	46.5 ± 3.1 d	33.4 ± 3.8 c	3.4 ± 0.1 b	3.8 ± 0.1 c

[a]Different letters in the same column shows that the values are significantly different at $P<0.05$.
[b]TDZ=2.27 μM, IAA =0.057 μM.
[c]TDZ=2.27 μM, NAA=0.054 μM.

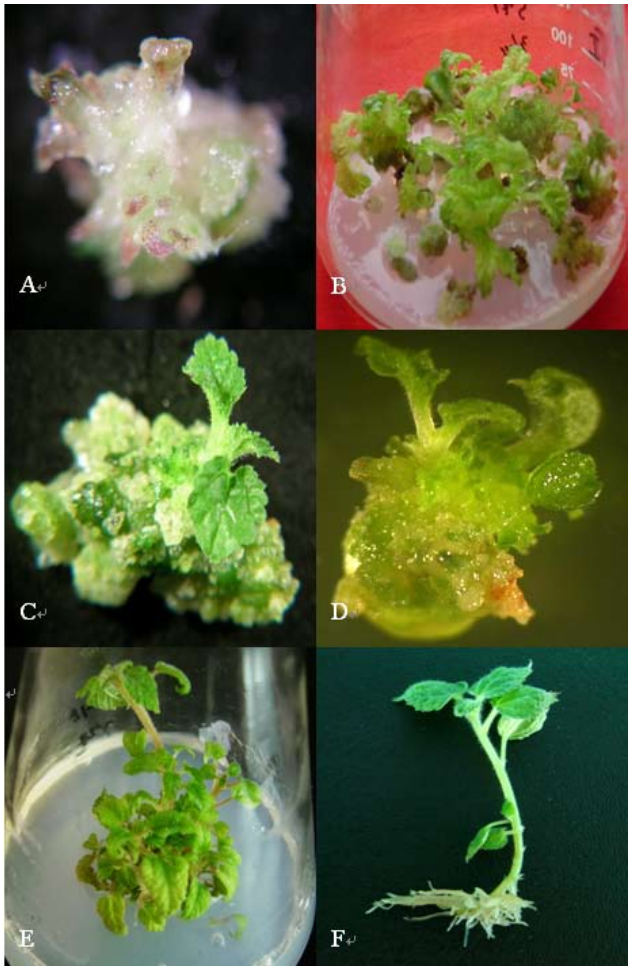

Figure 1. Plant regeneration from the cotyledons of ramie. (A) Shoot formation at the cutting edge of cotyledon 15 d after culture; (B) Regeneration of adventitious shoots from the cotyledons cultured for 8 weeks on MS medium with 2.27 μM TDZ and 0.057 μM IAA; (C) Induction of one shoot from a cotyledon 8 weeks after culture; (D) Multiple shoots regenerated from a cotyledon; (E) Elongated shoots on hormone-free MS medium; (F) A well-rooted plantlet.

selected randomly from the regenerated plantlets, which has never been conducted in the previous study on ramie tissue culture. Results showed that the regenerated plantlets demonstrated the fluorescence intensity similar to the control whose fluorescence intensity was preset to 50 (Figure 3A), which indicated that they were diploids (Figure 3B).

In conclusion, we report herein for the first time a protocol for highly efficient regeneration of plantlets from ramie cotyledon. The established regeneration system holds great potential for *Agrobacterium tumefacien*s-mediated transformation with the efforts of cultivar improvement for ramie, which is underway at present in our laboratory.

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Figure 2. A and B, Acclimatized plants in plastic pots containing soil, vermiculite and sand (2:1:1) in the greenhouse.

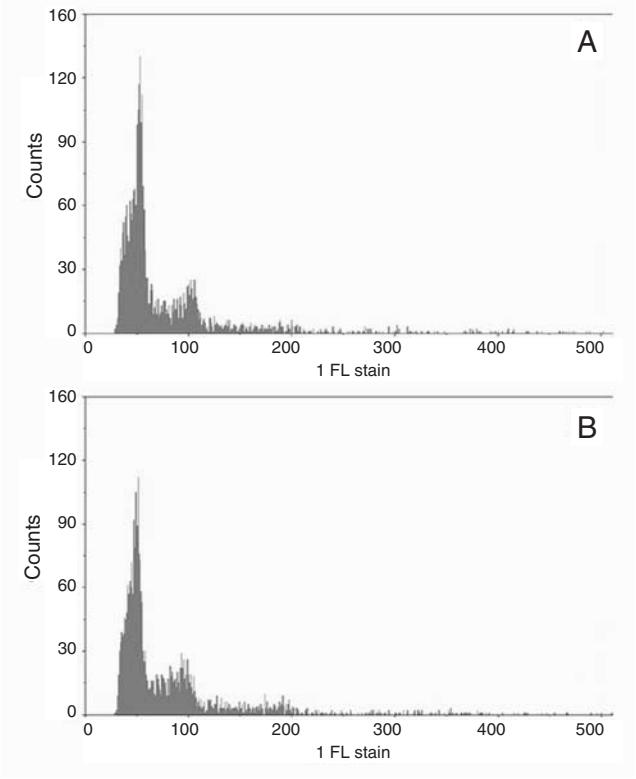

Figure 3. FCM histograms. (A) The diploid control; (B) One of the regenerated plantlets.

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噻苯隆（thidiazuron）誘導的苧麻高效不定芽再生體系的建立

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以苧麻子葉為外植體，對影響植株再生的因素進行了研究並建立了高效的不定芽再生體系。結果表明：取自發芽後培養 4 天的實生苗的子葉具有最高的再生頻率。在和噻苯隆（TDZ）搭配使用時，吲哚乙酸（IAA）是 IAA、吲哚丁酸（IBA）、奈乙酸（NAA）和 2,4-D 等四種生長素中效果最好的。不同濃度的 TDZ 和 IAA 配合使用獲得了不同的再生頻率，獲得最高再生頻率（86.2%）的培養基為附加 2.27 μM TDZ 和 0.057 μM IAA 的 MS 培養基。不同基因型的再生能力有顯著差別，在 6 個被測試的品種（材料）中，5041-3 的再生頻率最高。再生苗轉移到不含激素的培養基上進行伸長生長，長至 2 cm 左右時轉到含有 0.27 μM NAA 的 1/2MS 培養基上生根，生根後的植株有 4-5 片真葉時移栽到溫室中進一步生長。根據現有的報導，本研究是首次利用苧麻子葉建立高效再生體系，本研究結果將在苧麻的遺傳改良上發揮重要作用。

關鍵詞：器官發生；子葉；苧麻；thidiazuron。