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REGULAR ARTICLE

PROPAGATION OF *CLEOME SPINOSA* JACQ. THROUGH TISSUE CULTURE

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ABSTRACT

The tissue culture and rapid propagation of *Cleome spinosa* Jacq. was explored by investigating the effects of different plant growth regulators on callus induction, bud differentiation, and root formation of three types of explants. The results showed that hypocotyls and stem segments regenerated buds directly on growth regulator-free Murashige and Skoog (MS) medium. The highest callus induction rates of hypocotyls, stem segments, and leaves reached 100% and were obtained on the culture medium of MS + (1.0 to 2.0) mg/L kinetin (KT) + 0.02 mg/L α-naphthalene acetic acid (NAA), on which the leaves produced the best quality of calluses. The leaf-derived calluses were subcultured on MS + 0.5 mg/L KT +0.5 mg/L 6-benzylaminopurine (BAP) and achieved the highest differentiation rate of 100%, producing an average of 7.5 buds per explant. Inoculation with MS + 0.5 mg/L indole-3-butyric acid (IBA) resulted in the production of a number of thick roots by 66.7% of the regenerated buds. After transplanting, plantlets with more roots survived easily and grew well.

Keywords: *Cleome spinosa* Jacq., tissue culture, regeneration, plant growth regulator

INTRODUCTION

Cleome spinosa Jacq. is an annual and biennial herbaceous plant of the *Cleome* genus in Capparidaceae. This plant is native to the tropical Americas and currently widely cultivated worldwide (**Editorial committee of flora of china, 1979**). The raceme of this plant, with a long and sturdy pedicel, forms a lush, red, and white bouquet, highlighting it as a fair ornamental plant, which can be planted in flower beds and roadsides as well as cut as an ornamental plant and placed in a vase. The shoots and leaves of *C. spinosa* Jacq. are edible with a unique flavor. Eating the young shoots has a cooling effect and helps to expel the summer heat, relieving restlessness, quenching one's thirst and decreasing one's blood pressure. The essential oils of this plant have antioxidant, insecticidal, and antimicrobial effects (**McNeil *et al.*, 2010**). In addition, *C. spinosa* Jacq. also has strong tolerance to sulfur dioxide, as well as chlorine, and has a strong absorption capacity (**Du, 1986**) so that it beautifies the environment and purifies the air. Thus, this garden plant is worthy to be popularized.

C. spinosa Jacq., an important ornamental plant, relies mainly on seed propagation. However, seed propagation requires a long time with low germination percentage and demanding germination temperature, affecting the expansion of its cultivation area. By contrast, tissue culture is not subject to seasonal restrictions and can provide manual control of growing conditions needed for rapid propagation. **Miao *et al.* (1990)** used hypocotyls and cotyledon segments as explants in the tissue culture of *C. spinosa* Jacq. to study the effect of auxins, cytokinin, and gibberellic acid on callus induction, bud differentiation, and root formation. Suitable culture media were obtained, but they did not present specific values of differentiation and rooting rates. **Wang *et al.* (2004a, b)** also conducted a similar study. **Albarello *et al.* (2006)** used hypocotyls and stem segments as explants to determine suitable hormone combinations for micropropagation. However, different results were obtained in these studies because of the different hormones and explants used.

Mass production requirements are difficult to satisfy because of the limited and non-systematic reports on tissue culture of *C. spinosa* Jacq.. In this experiment, different explants, including hypocotyls, stem segments, and leaves, subjected to different combinations of growth regulators and concentrations were used to study the effects on regeneration to provide an efficient protocol for the mass propagation of *C. spinosa* Jacq. through tissue culture.

MATERIAL AND METHODS

Plant material

Seeds of *C. spinosa* Jacq. were harvested from plants cultivated in Sichuan Agricultural University. Mature seeds were first surface sterilized by 70% ethanol for 30 s, followed by 0.1% mercuric chloride for 8 min. The seeds were finally washed five times in sterilized distilled water. Sterilized seeds were inoculated on hormone-free MS medium (**Murashige and Skoog, 1962**).

Callus induction

The hypocotyl (1.0cm), stem segment (1.0cm) with a node, and leave segment (0.5cm×0.5cm) were excised when aseptic seedlings reached 10 cm high. These explants were inoculated on MS medium supplemented with varying combinations of 6-benzylaminopurine (BAP) (1, 2, 4, and 6 mg/L) or kinetin (KT) (1, 2, 4, and 6 mg/L) with 0.02 mg/L α-naphthalene acetic acid (NAA). The callus and bud induction rates were recorded for each treatment cultured for 30 d.

Multiple bud induction

The calluses induced from the leaves on medium of MS + 1.0 mg/L KT + 0.02 mg/L NAA were transferred to MS medium supplemented with varying combinations of BAP (1, 2, and 4 mg/L) and 0.5 mg/L NAA, or 0.5 mg/L BAP and 0.5 mg/L KT to induce buds. Bud induction rate and mean bud number were counted after 30 d of culture for each treatment.

Rooting culture

The regenerated multiple buds were divided into single buds (1.5 cm long), which then were inserted into the MS medium containing different concentrations of indole-3-butyric acid (IBA) (0, 0.5, and 1.0 mg/L) for rooting culture. Rooting rate and root number in each treatment were counted 15 d post-inoculation.

Culture condition and statistical analysis

The above culture media contained 30.0 g/L sugar and 6.0 g/L agar, with the pH adjusted to 5.8 before autoclaving at a pressure of 105 kPa for 20 min at 121 °C. All cultures were incubated under a 16 h photoperiod at 40 μmol/(m^2·s) light intensity at 25 °C ± 1 °C in 70% to 80% relative humidity. Each treatment was replicated thrice with 18 explants per replicate. Treatment means were compared using the data processing system (DPS) statistical software package (**Tang and Feng, 2002**) according to Duncan's multiple range test at 5% significance level.

Hardening and transplantation of plantlets

Rooted plantlets were washed with water to remove agar sticking to the roots, transferred to plastic pots containing a mixture of peat, sand and perlite (1:1:1 v/v/v), and then covered with a plastic membrane. After hardening, plantlets that have grown significantly were transferred to soil in the greenhouse.

RESULTS AND DISCUSSION

Primary culture

Table 1 shows that the highest callus induction rate of 100% was observed on the MS medium containing a combination of 1.0 or 2.0 mg/L KT with 0.02 mg/L NAA. All the hypocotyls, leaves, and stem segment explants induced calluses, among which those induced from leaves were the biggest and had the best quality. KT is more suitable for callus induction, consistent with the results obtained by **Wang *et al.* (2004b)**, but its concentration should not be excessively high. However, the hypocotyls and leaves failed to redifferentiate into buds in the presence of KT, and only some of stem segments regenerated buds.

For the media containing BAP, leaves on MS medium supplemented with 6.0 mg/L BAP and 0.02 mg/L NAA had the highest callus induction rate of 93.3%, followed by the stem segments at 86.7% on MS medium supplemented with 4.0 mg/L BAP and 0.02 mg/L NAA. There was a downward tendency trend in budding rate and mean bud number of explants with increasing concentrations of BAP.

For bud regeneration, regeneration rate of hypocotyls and stem segments on growth regulator-free MS medium was higher than on media with growth regulators and regeneration rates achieved by **Albarello *et al*. (2006)**. Moreover, budding time (3 days) on growth regulator-free MS medium was shorter. Buds regenerated directly from these two types of explants, and 2.4 to 2.7 buds per explant were observed. The directly regenerated buds took root and developed into normal plants, which can be used as a pathway for *in vitro* propagation. An advantage of direct organogenesis was the potential for maintaining genomic stability of regenerated plants (**Reddy *et al*., 1998**). Conversely, fewer leaves regenerated buds directly.

Table 1 Effects of different combinations of growth regulators on callus and bud induction

Culture medium (mg/L)	Callus induction rate (%)			Bud induction rate (%)		
	Hypocotyl	Leaf	Stem segment	Hypocotyl	Leaf	Stem segment
MS	26.7 d	6.7 e	13.3 f	90.0 a	6.7 a	100.0 a
MS + BAP 1.0 + NAA 0.02	66.7 b	73.3 c	26.7 e	33.3 b	6.7 a	73.3 b
MS + BAP 2.0 + NAA 0.02	53.3 bc	66.7 cd	53.3 d	26.7 bc	0 b	73.3 b
MS + BAP 4.0 + NAA 0.02	40.0 c	60.0 d	86.7 b	20.0 c	0 b	66.7 b
MS + BAP 6.0 + NAA 0.02	20.0 d	93.3 b	73.3 c	0 d	0 b	53.3 c
MS + KT 1.0 + NAA 0.02	100.0 a	100.0 a	100.0 a	0 d	0 b	6.7 e
MS + KT 2.0 + NAA 0.02	100.0 a	100.0 a	100.0 a	0 d	0 b	6.7 e
MS + KT 4.0 + NAA 0.02	66.7 b	93.3 b	66.7 c	0 d	0 b	46.7 c
MS + KT 6.0 + NAA 0.02	66.7 b	60.0 d	73.3 c	0 d	0 b	20.0 d

Legend: The means in the same column followed by different letters denote significantly different at the 5% level according to Duncan's multiple range test.

Multiple bud induction

After calluses induced from leaves were transferred to differentiation culture media, they first expanded, then regenerated into small buds in succession 10 d post-inoculation

(Figure 1-A), and finally developed into multiple buds. The most conducive media to bud differentiation of the calluses was MS + 0.5 mg/L BAP + 0.5 mg/L KT (Table 2), on which the induction rate was 100% and the mean bud number was 7.5, significantly higher than other treatments. The calluses regenerated fewer buds for a long time on MS medium and media supplemented with BAP and NAA. **Albarello *et al.* (2006)** observed callus formation at the basal end of leaf explants which failed to regenerate the media, perhaps because of different genotype of plant material or combinations of concentrations of growth regulators from ours.

Wang *et al.* (2004b) reported that MS + 2 mg/L BAP + 0.05 mg/L NAA was the best culture medium to induce buds for cotyledon-derived calluses. In our experiment, a low budding rate of calluses was obtained on the same culture medium, perhaps because the calluses were from different sources. However, a high budding rate with many buds was obtained on the MS medium supplemented with BAP and KT, probably because the independent use of cytokinins was more conducive to bud differentiation.

This initial dedifferentiation of explants into callus followed by the differentiation into adventitious buds can be used as another pathway for in vitro propagation. It should be noted that regeneration from callus may lead to genetic variation in regenerated plants (**Smith and Drew, 1990**).

Table 2 Effects of different combinations of growth regulators on bud induction of calluses

Culture medium (mg/L)	Bud induction rate (%)	Mean bud number
MS	5.6 c	0.06 c
MS + BAP 1.0 + NAA 0.05	61.0 b	0.61 b
MS + BAP 2.0 + NAA 0.05	66.0 b	0.66 b
MS + BAP 4.0 + NAA 0.05	61.0 b	0.61 b
MS + BAP 0.5 + KT 0.5	100.0 a	7.50 a

Rooting culture and transplantation

The rooting results in Table 3 shows that the same root induction rate of 66.7% was observed on the growth regulator-free MS medium and MS medium containing 0.5 mg/L IBA, but the mean root number was higher on the latter (Figure 1-B). High concentration of IBA (1.0 mg/L) resulted in low rooting rate. **Nassem and Jha (1994, 1997)** found that IBA was essential for root formation in *C. viscose* and *C. gynandra*. However, the use of auxins to

induce rhizogenesis *in vitro* of *C. spinosa* **(Albarello *et al*., 2006)** and *C. rosea* **(Simoes *et al*., 2004)** was not required. In the present study, although a certain concentration of IBA could increase the number of roots, it failed to improve rooting rate. Low rooting rate was perhaps due to the small buds. Hence, further research is needed to enhance rooting rate.

When the rooted plantlets reached a height of 5 cm, they were hardened and transferred to mixed substrates with 62.5% survival rate. Plantlets with thicker roots survived easily and developed into morphologically normal plants.

Table 3 Effects of different culture media on root induction

Culture medium (mg/L)	Root induction rate (%)	Mean root number
MS	66.7 a	5.3 b
MS + IBA 0.5	66.7 a	11.6 a
MS + IBA 1.0	50 b	5.8 b

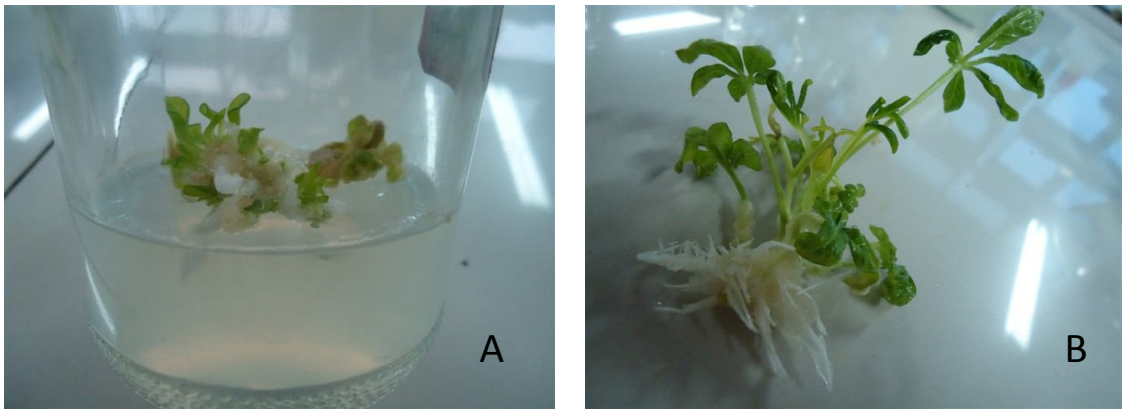

Figure 1 *In vitro* regeneration of *Cleome spinosa* Jacq. A: Bud induction from calluses. B: Rooted plantlets

CONCLUSION

In summary, hypocotyl and stem segments of *C. spinosa* Jacq. can be used as explants and cultured on MS medium without plant growth regulators. These explants can produce buds after 3 d inoculation and take roots. This pathway of propagation takes a short time, but the propagation coefficient is not high and can only produce 2 to 3 buds per explant. Another pathway has a high propagation coefficient but takes a relatively long time. Leaves are first cultured on MS + 1.0 mg/L KT + 0.02 mg/L NAA to induce callus, then transferred to MS +

0.5 mg/L KT +0.5 mg/L BAP to induce buds, and finally take roots. In production practice, an appropriate propagation method can be selected according to different purposes.

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