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ORIGINAL ARTICLE

Callus induction and high-efficiency plant regeneration via somatic embryogenesis in *Papaver nudicaule* L., an ornamental medicinal plant

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Abstract We describe culture conditions for a high-efficiency in vitro regeneration system of *Papaver nudicaule* through somatic embryogenesis and secondary somatic embryogenesis. The embryogenic callus induction rate was highest when petiole explants were cultured on Murashige and Skoog (MS) medium containing 1.0 mg l^{-1} α-naphthaleneacetic acid (NAA) and 0.1 mg l^{-1} 6-benzyladenine (BA) (36.7%). When transferred to plant growth regulator (PGR)-free medium, 430 somatic embryos formed asynchronously from 90 mg of embryogenic callus in each 100-ml flask. Early-stage somatic embryos were transferred to MS medium containing 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA to germinate at high frequency (97.6%). One-third-strength MS medium with 1.0% sucrose and 1.0 mg l^{-1} GA_3 had the highest frequency of plantlet conversion from somatic embryos (91.2%). Over 90% of regenerated plantlets were successfully acclimated in the greenhouse. Secondary somatic embryos were frequently induced directly when the excised hypocotyls of the primary somatic embryos were cultured on MS medium without PGRs. Sucrose concentration significantly affected the induction of secondary embryos. The highest induction rate (89.5) and number of secondary somatic embryos per explant (9.3) were obtained by 1% sucrose. Most secondary embryos (87.2–94.3%) developed into the cotyledonary stage on induction medium. All cotyledonary secondary embryos were converted into plantlets both in liquid and on semisolid 1/3-strength MS medium with 1.0% sucrose.

Keywords Papaveraceae · Germination · Embryogenic callus · Somatic embryo · Secondary somatic embryo

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Introduction

Papaver nudicaule L. (Iceland poppy), a member of the family Papaveraceae, is a perennial or biennial that is native to the arctic regions of Eurasia and North America. *P. nudicaule* is generally used as an ornamental plant because of its wide range of pastel flower colors. The whole plants and capsules of native species have also been widely used as antitussive, analgesic and anti-diarrheal drugs in traditional Chinese medicine; the plants accumulate several alkaloids, and the main active constituent is known as nudicauline (Istatkova et al. 2008; Lan et al. 2006; Philipov et al. 2007; Zhang et al. 1997). Recently, its demand has been increasing for use in the pharmaceutical industry; for example, *P. nudicaule* extracts are used as raw materials for production of the cough medicine

"Kechuanning capsules". Over-exploitation has led to a rapid decline of *P. nudicaule* in nature. To promote wildlife conservation and to meet the needs of production, methods to optimize the artificial cultivation of *P. nudicaule* have recently been started in China (Zhen et al. 2006).

P. nudicaule is commonly propagated by seeds. However, cultivated seed populations are rarely homogeneous. High flower quality and good agronomic plant performance need to be improved by combining traditional breeding methods with updated propagation protocols. Development of an advanced propagation technology such as somatic embryogenesis might be an efficient method for biotechnological improvement of *P. nudicaule*. Somatic embryogenesis is a desirable method for genetic improvement because of its high multiplication potential and low risk of chimera development (Stasolla and Yeung 2003). In vitro propagation of *P. nudicaule* is just emerging, and few reports are available. *P. nudicaule* plants can be generated through in vitro seed culture (Cheng et al. 2001) and shoot multiplication (Savona et al. 2001). In addition, a cell culture method has been used for *P. nudicaule* to produce alkaloids, but no regenerated plants were obtained (Yu et al. 2003). Somatic embryogenesis and plant regeneration of *P. nudicaule* has not yet been described, although these methods have been reported for other *Papaver* species, such as *Eschscholzia californica* (Park and Facchini 1999), *P. somniferum* (Nessler 1982), *P. orientale* (Iiahi and Ghauri 1997), *Hylomecon vernalis* (Kim et al. 2003) and *Chelidonium majus* (Kim et al. 1999). In this study, we describe *P. nudicaule* culture conditions that result in a high frequency of somatic embryogenesis and plant regeneration from leaf and petiole explants.

Materials and methods

Plant material

Seeds of *P. nudicaule* L. were collected from Hailaer, Inner Mongolia, P.R. China, and stored at 4°C. The seeds were agitated in 70% ethanol for 1 min and surface-sterilized using 1.0% sodium hypochlorite solution with 0.02% (v v^{-1}) Tween 20 for 10 min, then rinsed five times with sterile distilled water. The surface-sterilized seeds (an average of 30 seeds in a flask) were spread on MS (Murashige and Skoog 1962) agar medium with 3.0% sucrose. Leaf and petiole explants from 7-day-old seedlings were cut transversely into 0.5-cm-long sections and cultured for somatic embryo induction.

Induction and proliferation of embryogenic callus

The induction medium was MS agar medium containing 3.0% sucrose supplemented with 0.1 or 1.0 mg l^{-1} of α-naphthaleneacetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D), either alone or in combination with 6-benzyladenine (BA) at 0.1 or 1.0 mg l^{-1}. Five explants were cultured in each flask with ten replicates. The percentage of embryogenic callus induction was recorded after 8 weeks of culture.

To promote proliferation, the friable embryogenic callus was separated from primary explants and maintained on initiation medium under darkness, subculturing at 3-week intervals. Approximately 90 mg of embryogenic callus was cultured in a flask with five replications. After subculturing three times, the embryogenic callus multiplication rate [(final fresh weight − initial fresh weight)/initial fresh weight] was calculated.

Development of somatic embryos

Friable, fast-growing embryogenic calluses were selected and transferred to agar-solidified MS medium containing 3.0% sucrose without plant growth regulators (PGRs). To stimulate the germination of somatic embryos, after 2 weeks of culture on PGR-free medium, embryo populations (mainly torpedo-shaped embryos) from a flask were transferred to a new flask containing MS medium with 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA. Somatic embryo formation efficiency was determined by counting the number of embryogenic callus-producing somatic embryos per flask. The germination frequency was recorded after 4 weeks of culture.

Plant regeneration

Germinated somatic embryos were transferred to various strengths of MS agar medium (1/4-, 1/3-, 1/2-, full-, or double-strength) with 3.0% (w v^{-1}) sucrose without PGRs. The culture medium was further optimized by transferring germinated somatic embryos to 1/3-strength MS medium containing different concentrations of sucrose (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, or 6.0%) without PGRs. The effect of GA_3 on germination was also investigated by counting the number of germinated somatic embryos cultured on 1/3-strength MS medium containing 1.0% sucrose and supplemented with various concentrations of GA_3 (0.1, 0.5, 1.0, or 3.0 mg l^{-1}). Six germinated somatic embryos were cultured in a flask with five replications. The percentage of plantlet regeneration was collected after 4 weeks of culture.

Transplantation

Sixty plantlets regenerated in vitro with well-developed leaf and root systems were selected and then transferred to pots containing autoclaved sand and soil (1:3 mixture).

Pots were covered with polythene bags to maintain high humidity. The bags were perforated and the covers were removed after 2 weeks when the plants showed new leaves. After 5 weeks of hardening, the surviving plants were transferred to the greenhouse.

Secondary somatic embryogenesis

During somatic embryogenesis, we found that the secondary somatic embryos frequently formed from the germinated somatic embryos in the medium without PGRs. To evaluate the effect of sucrose concentration on secondary somatic embryo formation, hypocotyl explants excised from germinated somatic embryos were cultured in MS medium with 1.0, 3.0, or 5.0% sucrose. Five hypocotyl segments were cultured in a flask with ten replications. After 8 weeks, the secondary somatic embryo induction rate and the number of embryos per explant were counted. Then, induced secondary somatic embryos were transferred to 1/3-strength MS agar medium or liquid medium containing 1.0% sucrose without PGRs. The plantlet regeneration efficiency was calculated after 4 weeks.

Culture conditions and statistical comparison

Media used in experiments were adjusted to pH 5.8 before adding 0.8% plant agar (Duchefa, Netherland), and then sterilized by autoclaving at 1.1 kg cm^{-2} (121°C) for 15 min. Cultures were performed in 100-ml Erlenmeyer flasks with 30 ml medium and subcultured at 4-week intervals, and were maintained at 24 ± 2°C with a 16-h/day photoperiod using cool white fluorescent tubes (36 μmol s^{-1} m^{-2}). Percentage data were arcsine-transformed prior to analysis. Means were separated using Duncan's multiple range test at $P = 0.05$.

Results and discussion

Embryogenic callus induction and multiplication

Callus formation was observed from leaf and petiole explants after they were cultured on the callus induction medium for 3 weeks. The compound 2,4-D was more effective than NAA for callus induction when used alone or in combination with BA (data not shown). However, somatic embryos were not formed from explants cultured on medium containing 2,4-D. Consequently, numerous adventitious roots were induced on the surface of all calluses. When cultured on medium containing NAA and BA (Fig. 1a), friable, yellowish-white embryogenic calluses formed at the cut edges of explants and spread to all surfaces of cultured explants within 4–6 weeks. Petiole explants produced more embryogenic calluses than leaf explants. These results showed that relatively high NAA and low BA concentrations were crucial for high-frequency embryogenic callus induction of *P. nudicaule* (Table 1). The optimal medium composition for embryogenic callus induction was MS medium supplemented with 1.0 mg l^{-1} NAA and 0.1 mg l^{-1} BA with an average of 36.7% petiole explants producing an embryogenic callus. The embryogenetic callus developed on medium containing 1.0 mg l^{-1} NAA and 0.1 mg l^{-1} BA was characterized by a higher multiplication index (3.9) (Table 2).

The use of NAA in combination with the cytokinin to induce somatic embryogenesis has already been reported for other related species such as *E. californica* (Park and Facchini 1999) and *P. orientale* (Iiahi and Ghauri 1997). For *E. californica* (Park and Facchini 1999), calluses were induced on medium supplemented with 2,4-D; this primary callus was then transferred to medium containing NAA and BA to establish an embryogenic callus and promote somatic embryogenesis.

The type of explants also has a great effect on the induction of somatic embryogenesis (Pedroso and Pais 1995). In the present study, embryogenic callus induction frequency on petiole explants was over fourfold higher than on leaf explants, indicating that petiole explants are more suitable for embryogenic callus induction than leaf explants. This kind of physiological response has been observed in other Papaveraceae species, such as *H. vernalis* (Kim et al. 2003).

Development of somatic embryos and plant regeneration

Numerous globular somatic embryos formed 7–10 days after friable embryogenic calluses (Fig. 1a) were transferred to MS medium containing 3.0% sucrose without PGRs (Fig. 1b). Continued formation of globular embryos and embryo germination occurred in this medium. Once induced, some globular embryos spontaneously germinated through the heart, torpedo and cotyledonary stages (Fig. 1c, d). Then, somatic embryos developed rapidly, although the process was asynchronous. Ultimately, an average of 430 somatic embryos were harvested from approximately 90 mg of embryogenic callus in a 100-ml Erlenmeyer flask within 4 weeks, including 94 (21.8%) germinated embryos (Table 3; Fig. 1d, e). Plantlet conversion occurred when germinated somatic embryos were transferred to regeneration medium (data not shown). One-third-strength MS medium with 1.0% sucrose had the highest frequency of plantlet conversion; however, the conversion frequency was very low (12.4%).

When embryo populations that had been developed on medium without PGRs after a 2-week culture were

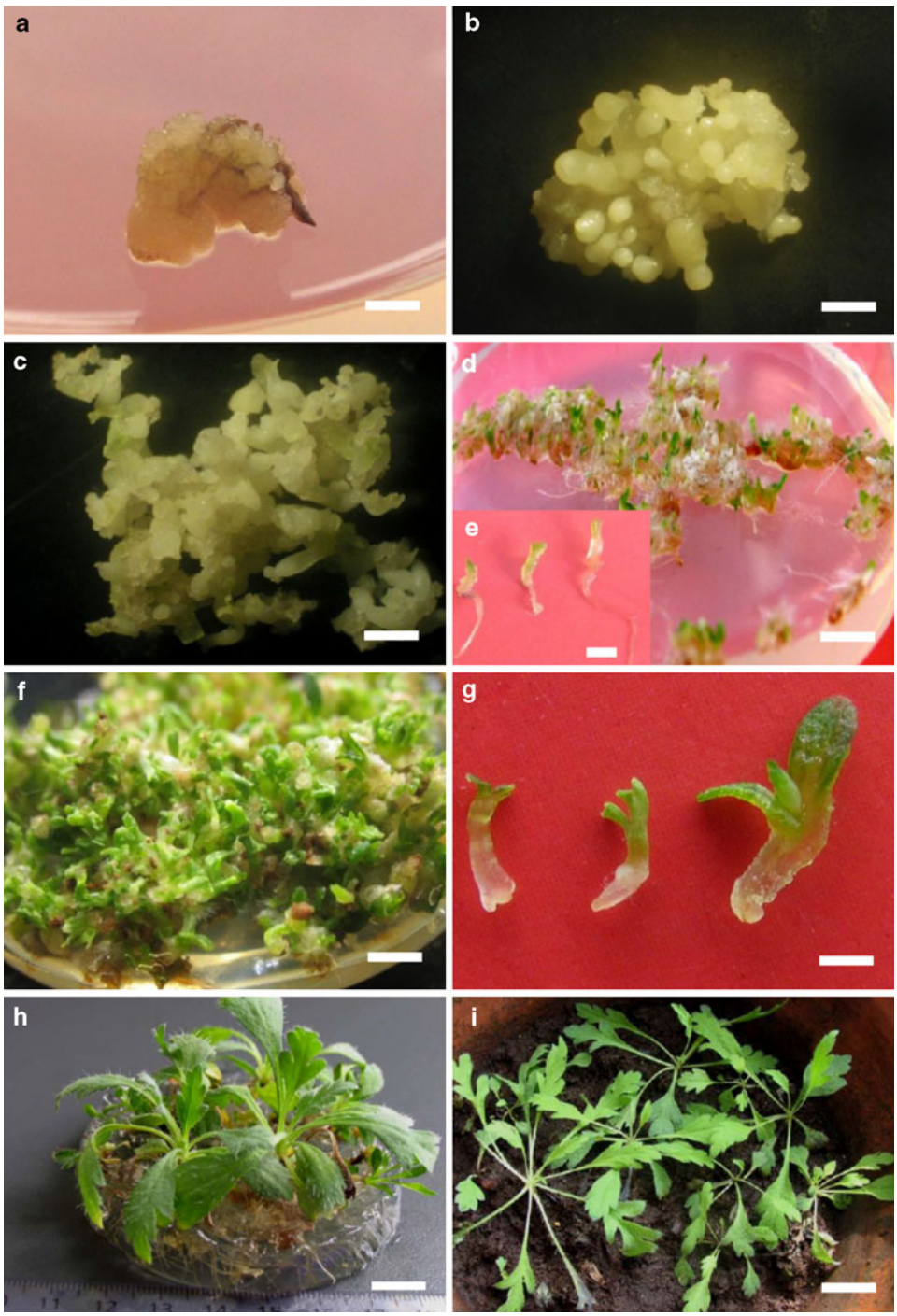

Fig. 1 In vitro regeneration of *P. nudicaule* via somatic embryogenesis. **a** Friable embryogenic callus induced from petiole explants on MS medium supplemented with 1.0 mg l^{-1} NAA and 0.1 mg l^{-1} BA (*bar* 2 mm). **b** Early-stage somatic embryos induced when embryogenic callus was transferred to MS medium containing 3.0% sucrose without PGRs (*bar* 0.5 mm). **c** Torpedo- and cotyledonary-stage somatic embryos developed on MS medium containing 3.0% sucrose without PGRs (*bar* 1 mm). **d** Somatic embryos germinated on MS medium containing 3.0% sucrose without PGRs (*bar* 6 mm). **e** Closed-up view of germinated somatic embryos on MS medium containing 3.0% sucrose without PGRs (*bar* 2 mm). **f** Somatic embryos germinated on MS medium containing 1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA (*bar* 6 mm). **g** Closed-up view of germinated somatic embryos on MS medium containing 1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA (*bar* 1.5 mm). **h** Regenerated plantlets 1 month after cultured on 1/3X-strength MS medium without PGRs (*bar* 10 mm). **i** Plant development following transplantation of regenerated plantlets into a sand/soil mixture and growth under greenhouse conditions (*bar* 16 mm)

transferred to MS medium containing 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA, most embryos (409.7, 97.6%) germinated within 2 weeks (Table 3; Fig. 1f). The structure of germinated somatic embryos that developed on the medium containing 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA differed from those grown on medium without PGRs. Germinated somatic embryos from the former medium had well-developed apical buds and thickened cotyledons and hypocotyls (Fig. 1 g) compared with the latter, which had slender hypocotyls, less-developed apical buds and elongated primary roots (Fig. 1e). Primary leaves and roots were induced within 1 week after germinated somatic embryos were transferred to regeneration medium at low density. Regenerated plantlets with well-developed shoots and roots (Fig. 1 h) were transferred to a mixture of sand and soil (1:3). Approximately 91% of the plants survived without wilting after 4 weeks of acclimation, and they successfully grew into fertile plants (Fig. 1i).

Table 1 Effect of NAA and BA on callus and embryogenic callus induction frequency from *P. nudicaule* leaf and petiole explants after 6 weeks of culture

Explants	PGRs (mg l^{-1})	Embryogenic callus induction (%)[a]
Leaf	NAA 0.1	0
	NAA 0.1 + BA 0.1	0
	NAA 0.1 + BA 1.0	0
	NAA 1.0	0
	NAA 1.0 + BA 0.1	8.3 c
	NAA 1.0 + BA 1.0	6.7 c
Petiole	NAA 0.1	0
	NAA 0.1 + BA 0.1	0
	NAA 0.1 + BA 1.0	6.7 c
	NAA 1.0	0
	NAA 1.0 + BA 0.1	36.7 a
	NAA 1.0 + BA 1.0	20.0 b

[a] Means followed by the same letters are not significantly different using Duncan's multiple range test at $P = 0.05$

Table 2 Effect of NAA and BA on multiplication of the embryogenic callus of *P. nudicaule* after 4 weeks of culture

PGRs (mg l^{-1})	Initial fresh weight of embryogenic callus (mg)[a]	Embryogenic callus multiplication rate (times)
NAA 0.1 + BA 1.0	93.7 a	1.4 c
NAA 1.0 + BA 0.1	92.1 a	3.9 a
NAA 1.0 + BA 1.0	87.5 a	3.1 b

[a] Means followed by the same letters within a column are not significantly different using Duncan's multiple range test at $P = 0.05$

Table 3 Effect of BA and NAA on germination of *P. nudicaule* somatic embryos in 100-ml Erlenmeyer flasks after 4 weeks of culture

Treatment	Initial fresh weight of embryogenic callus (mg)	Somatic embryos per flask	Germinated embryos per flask[a]	Germination frequency (%)
T1	91.8	430.3	94.0 b	21.8 b
T2	90.3	419.7	409.7 a	97.6 a

T1 Embryogenic callus cultured on MS medium with 3.0% sucrose for 4 weeks; *T2* cultured on MS medium with 3.0% sucrose for 2 weeks, then transferred to MS medium with 3.0% sucrose containing 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA

[a] Means followed by the same letters within a column are not significantly different using Duncan's multiple range test at $P = 0.05$

The success of large-scale micropropagation through somatic embryogenesis relies on plantlet conversion from embryos. A low conversion rate is one of the most common problems for somatic embryogenesis. According to Stasolla and Yeung (2003), the low conversion rate may be a consequence of poor polarization during apical meristem development. In our experiments, BA and NAA treatment significantly increased the regeneration ability of somatic embryos, which seemed to be obtained not only by increasing the fresh weight and length of embryonic axes of germinated embryos but also by facilitating the symmetry development of bipolar structures through the stimulation of shoot apical meristem development and delay of the root meristem growth. Similar results concerning the effect of BA alone and in combination with other PGRs on increasing the conversion rate were reported in *E. californica* (Park and Facchini 1999) and peach palm (Steinmacher et al. 2007).

Table 4 Effect of basal salt on conversion frequency and plant growth for *P. nudicaule* somatic embryos on medium with 3.0% sucrose after 4 weeks of culture

MS medium strength	Conversion (%)[a]	Shoot length (mm)	Root number per plantlet
1/4	53.2 ab	15.1 a	12.1 a
1/3	65.2 a	15.8 a	14.7 a
1/2	61.2 a	16.9 a	14.5 a
1	40.7 b	13.3 a	9.3 b
2	16.2 c	2.1 b	2.8 c

[a] Means followed by the same letters within a column are not significantly different using Duncan's multiple range test at $P = 0.05$

Among the various MS medium strengths used in the experiment, the conversion frequency was apparently the highest on 1/3-strength MS medium, although the data were not significantly different among 1/4-, 1/3- and 1/2-strength MS media. Similarly, the shoot growth and root number was significantly higher on 1/3-strength and 1/2-strength MS media compared with other strength MS media. In contrast, medium strength above full strength was inhibitory (Table 4). Based on these data, 1/3-strength MS medium was selected for a further study to optimize the sucrose concentration on the conversion medium. The result indicated that a low concentration of sucrose (0.5–2.0%) increased the frequency of somatic embryo conversion and the growth of shoots and roots; the optimal sucrose concentration was 1.0% (Table 5). Similar observations have been reported for *E. californica* (Park and Facchini 1999). Supplementation with GA_3 in the conversion medium had a positive effect on the rate of somatic embryo conversion and further growth of shoots, whereas the average number of roots decreased. Conversion frequency was highest on medium supplemented with 1.0 mg l^{-1} GA_3 (Table 6). It is known that GA_3 is especially necessary to culture somatic embryos that undergo dormancy (Choi et al. 1999). The significant stimulatory effect of GA_3 was proven by culturing *E. californica* (Park and Facchini 1999) and *Eleuthecocos senticosus* (Choi et al. 1999). On the other hand, a negative effect of GA_3 on

Table 5 Effect of sucrose concentration on conversion into plantlets from somatic embryos and growth of *P. nudicaule* plantlets on 1/3-strength MS medium after 4 weeks of culture

Sucrose (%)	Conversion (%)[a]	Shoot length (mm)	Root number per plantlet
0.5	68.8 b	19.2 a	17.3 a
1.0	81.2 a	22.3 a	19.8 a
2.0	75.2 ab	20.7 a	18.1 a
3.0	65.2 bc	16.9 ab	14.7 b
4.0	55.2 c	9.2 b	8.3 c
5.0	10.6 d	3.8 c	1.0 d
6.0	0 e	–	–

[a] Means followed by the same letters within a column are not significantly different using Duncan's multiple range test at $P = 0.05$

Table 6 Effect of GA_3 on conversion frequency and plant growth for *P. nudicaule* somatic embryos on 1/3–strength MS medium with 1% sucrose after 4 weeks of culture

GA_3 (mg l^{-1})	Conversion (%)[a]	Shoot length (mm)	Root number per plantlet
0	81.2 b	22.3 b	19.8 a
0.1	89.3 ab	23.6 b	14.8 b
0.5	83.7 ab	26.5 a	15.3 b
1.0	91.2 a	30.9 a	12.1 c
3.0	86.3 ab	30.4 a	8.7 d

[a] Means followed by the same letters within a column are not significantly different using Duncan's multiple range test at $P = 0.05$

conversion and further growth also occurs if the concentration is too high (Park and Facchini 1999). In our study, inclusion of GA_3 on regeneration medium had a positive effect on improving the conversion rate of germinated somatic embryos.

Secondary somatic embryogenesis

When the explants were cultured in secondary somatic embryo induction medium, they began to enlarge slightly after day 4 or 5. After about 10 days of culture, the somatic embryos were slightly brown. In the following 6 weeks, secondary somatic embryos were induced directly from the hypocotyl explants (Fig. 2a), and the results indicated that the effect of sucrose concentration on the induction rate and the number of secondary embryos per explant was significant; the highest induction rate was obtained by 1% sucrose. The highest rate of secondary somatic embryo induction and the highest number of embryos per explant were 89.5 and 9.3%, respectively (Table 7). Moreover, almost all of the induced secondary embryos grew into cotyledonary embryos. Direct secondary somatic embryogenesis on PGR-free medium has been observed in several species, including black pepper (Nair and Dutta Gupta 2005) and Horse chestnut (Calic et al. 2005), and is desirable as it enhances multiplication potential and maintenance of the somatic embryo culture (Raemakers et al. 1995).

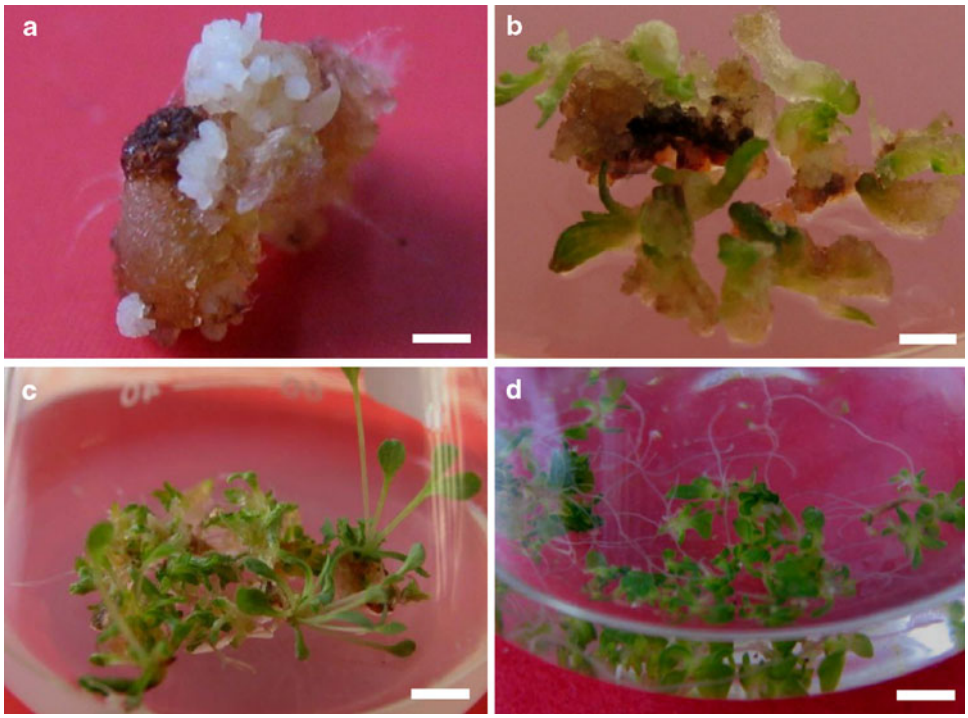

Fig. 2 Secondary somatic embryogenesis and plantlet regeneration from hypocotyl explants of primary somatic embryos of *P. nudicaule*. **a** Secondary somatic embryos induced directly from hypocotyl explants of primary somatic embryos after 8 weeks in culture (*bar* 0.5 mm). **b** Germinated secondary somatic embryos (*bar* 1 m). **c** Secondary somatic embryos regenerated into plantlets on agar medium (*bar* 10 mm). (*bar* 7 mm). **d** Secondary somatic embryos regenerated into plantlets in liquid medium (*bar* 6 mm)

Table 7 Effect of sucrose concentration on secondary somatic embryogenesis and embryo maturation of *P. nudicaule* on MS medium after 4 weeks of culture

Sucrose (%)	Secondary somatic embryogenesis (%)[a]	Secondary somatic embryos per explant	Cotyledonary embryos (%)
1.0	89.5 a	9.3 a	90.4
3.0	49.1 b	4.6 b	94.3
5.0	38.5 b	5.1 b	87.2

[a] Means followed by the same letters within a column are not significantly different using Duncan's multiple range test at $P = 0.05$

When transferred to the 1/3-strength MS agar medium, the induced secondary somatic embryos germinated within 1 week (Fig. 2b), and all cotyledonary embryos developed into plantlets within 4 weeks (Fig. 2c). In liquid medium, all secondary somatic embryos germinated after 4–5 days of culture and regenerated into intact plantlets at day 7 (Fig. 2d). This might make it possible to promote cyclic secondary somatic embryogenesis and be used as a genetic transformation or mass propagation tool for *P. nudicaule*.

In conclusion, we report a high-efficiency in vitro regeneration system of *P. nudicaule* through somatic embryogenesis and secondary somatic embryogenesis. The protocol established in this study maybe helpful not only for the large scale of vegetative propagation but also for genetic improvement of *P. nudicaule* through transformation studies.

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References

Calic D, Zdravkovic-Korac S, Radojevic L (2005) Secondary embryogenesis in androgenic embryo cultures of *Aesculus hippocastanum* L. Biol Plant 49:435–438

Cheng HX, Yu RM, Zhang H, Ma L, Yao XS (2001) Seedling culture and callus induction of *Papaver nudicaule* L. J Shenyang Pham Univ 18:128–130

Choi YE, Kim JW, Yoon ES (1999) High frequency of plant production via somatic embryogenesis from callus or cell suspension cultures in *Eleutherococcus senticosus*. Ann Bot 83:309–314

Iiahi I, Ghauri EG (1997) Regeneration in cultures of *Papaver bracteatum* as influenced by growth hormones and temperature. Plant Cell Tiss Org Cult 38:81–83

Istatkova R, Philipov S, Yadamsurenghiin GO, Samdan J, Dangaa S (2008) Alkaloids from *Papaver nudicaule* L. Nat Prod Res 22:607–611

Kim SW, Min BW, Liu JR (1999) High frequency plant regeneration from immature ovule-derived embryogenic cell suspension cultures of *Chelidonium majus* var. asiaticum. Plant Cell Tiss Org Cult 56:125–129

Kim SW, In DS, Kim TJ, Liu JR (2003) High frequency somatic embryogenesis and plant regeneration in petiole and leaf explant cultures and petiole-derived embryogenic cell suspension cultures of *Hylomecon vernalis*. Plant Cell Tiss Org Cult 74:163–167

Lan R, Cui J, Qin XY, Tang L, Yang RM (2006) The chemical research and application exploiture of *Papaver nudicaule*. Stud Trace Elem Health 23:51–53

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Nair RR, Dutta Gupta S (2005) High-frequency plant regeneration through cyclic secondary somatic embryogenesis in black pepper (*Piper nigrum* L). Plant Cell Rep 24:699–707

Nessler CL (1982) Somatic embryogenesis in the opium poppy, *Papaver somniferum*. Physiol Plant 55:453–458

Park SU, Facchini PJ (1999) High-efficiency somatic embryogenesis and plant regeneration in California poppy, *Eschscholzia californica* Cham. Plant Cell Rep 19:421–426

Pedroso MC, Pais MS (1995) Factors controlling somatic embryogenesis. Plant Cell Tiss Org Cult 43:147–154

Philipov S, Istatkova R, Yadamsurenghiin GO, Samdan J, Dangaa S (2007) A new 8, 14-dihydropromorphinane alkaloid from Papaver nudicaule L. Nat Prod Res 21:852–856

Raemakers CJJM, Jacobsen E, Visser RGF (1995) Secondary somatic embryogenesis and applications in plant breeding. Euphytica 81:93–107

Savona M, Semeria L, Allavena A, Carli S, Profumo P (2001) In vitro culture of *Papaver nudicaule* L. Acta Hortic 560:469–472

Stasolla C, Yeung EC (2003) Recent advances in conifer somatic embryogenesis: improving somatic embryo quality. Plant Cell Tiss Org Cult 74:15–35

Steinmacher DA, Clement CR, Guerra MP (2007) Somatic embryogenesis from immature peach palm inflorescence explants: towards development of an efficient protocol. Plant Cell Tiss Org Cult 89:15–22

Yu RM, Wang Y, Ma L, Zhang H, Yao XS (2003) Production of active alkaloids by elicited *Papaver nudicaule* cell suspension cultures. Pharm Biotechnol 10:218–222

Zhang YJ, Yu YF, Kang SW (1997) Studies on the chemical constituents of capsule of wild poppy (*Papaver nudicaule*). Chinese Traditional and Herbal Drugs 28:7–10

Zhen ZX, Li YX, Na SZ, Yang GM, Zhang DH (2006) Preliminary study of manual planting and transplanting technology of *Papaver nudicaule*. Seed 25:1–6