Shoot organogenesis and plant regeneration from leaf and petiole explants of *Corydalis saxicola* Bunting

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Abstract

Corydalis saxicola Bunting, a medicinal herb known for alkaloids, grows mainly in Guizhou province and northwest Guangxi province of China. In this study, for the first time for this plant, an efficient adventitious shoot proliferation and regeneration system *via* shoot organogenesis from leaf and petiole explants was established. Cytokinins, including 6-benzyladenine (BA) and thidiazuron (TDZ), induced callus and then shoot organogenesis. Most adventitious shoots were induced from leaf explants on Murashige and Skoog (MS) medium (pH 6.5) supplemented with 2.0 µM BA and 0.5 µM α-naphthaleneacetic acid (NAA). This medium was also optimal for shoot proliferation. The rooting ½MS medium was supplemented with 1.0 µM indole-3-butyric acid (IBA) and 0.2 µM NAA; 95.7% of shoots induced roots within 30 d and 91% of plantlets survived on a peat:vermiculite (1:1, v:v) substrate after 90 d.

Keywords *Corydalis saxicola* · Leaf · Petiole · Adventitious shoot · pH value · Shoot proliferation · Regeneration · Rooting · Transplantation

Introduction

The genus *Corydalis* DC. (Fumariaceae) includes 428 species of herbaceous plants (Jiang *et al*. 2018). The geographic center of this genus is the Sino-Himalayan region, although naturalized plants can be found throughout north temperate areas and parts of eastern Africa (Wu *et al*. 1996). China has 298 endemic *Corydalis* species, many of which are cultivated as garden ornamentals (Wen *et al*. 1993).

Corydalis saxicola Bunting, a perennial herb, is found in Guizhou, Yunnan, Sichuan, and other provinces of southwest China but is mainly located in Guizhou and northwest Guangxi in China (Wen *et al*. 1993). It is a traditional folk medicine in southwest China and is clinically used for the treatment of liver diseases, such as hepatitis B, cirrhosis, and liver cancer (Li *et al*. 2010; Dai *et al*. 2018; Liu *et al*. 2018). Studies have shown that it is rich in alkaloids, steroids, triterpenes, flavonoids, nucleosides, and other compounds, although alkaloids are the main group of compounds (Wu *et al*. 2007; Guo *et al*. 2021; Xie *et al*. 2021). Most of these have considerable biological activities, such as antiviral, anti-inflammatory, anti-tumor, hepatoprotective, bacteriostatic, inhibition of acetylcholinesterase, analgesia, and other activities, especially for the treatment of hepatitis B, liver cancer, and cirrhosis, as well as other diseases (Kuai *et al*. 2020; Ju *et al*. 2021; Zhang *et al*. 2022). Consequently, the ability to mass produce this plant would fortify its expanded use and application.

Even though *C. saxicola* has high medicinal value and thus considerable economic benefits, resource output is sparse. This limitation is compounded by harsh natural growth habitats, low reproductive output, and difficulties in establishing large stands or populations (Xie *et al*. 2021). Consequently, large pools of natural stands and reserves of *C. saxicola* are very limited. In order to satisfy commercial demand without negatively impacting natural stands, there is an urgent need to carry out research to develop a biotechnology-based all-year-round industrial system of cultivation. Even though *C. saxicola*

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can produce seeds, increasing to about 26% after seeds were stored in sealed paper bags at low temperatures (3 to 6 °C), and since there is no dormancy period, the optimal sowing period is between April and August (Jiang *et al.* 2006). Propagation by seeds is, thus, not a reliable or sustainable method.

A few tissue culture studies for *C. saxicola* have been published. Callus induction and cell suspension cultures of *C. saxicola* were established to harvest total alkaloids on modified B5 medium (pH 6.0) with 0.5 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 2 mg L^{-1} 6-benzyladenine (BA), which was the most favorable medium for callus induction, with the highest induction rate (approximately 95%) and greatest fresh weight of callus (1.7 g per leaf explant) (Cheng *et al.* 2006). Shoot tips were cultured on Murashige and Skoog (MS; Murashige and Skoog 1962) medium to test the effects of multiple combinations of plant growth regulators (PGRs) on the induction of axillary shoots and rooting induction (Wei *et al.* 2014). It was observed that MS medium with 0.5 mg L^{-1} BA and 0.2 mg L^{-1} indole-3-acetic acid was the optimal induction medium for axillary shoots while 100% rooting was observed in ½MS with 0.5 mg L^{-1} indole-3-butyric acid (IBA), 0.3 mg L^{-1} α-naphthaleneacetic acid (NAA), and 0.5 g L^{-1} active charcoal, with 84% transplant survival within 30 d (Wei *et al.* 2014). In another study that used MS medium with 0.2 mg L^{-1} thidiazuron (TDZ) and 0.1 mg L^{-1} NAA, as well as other PGR combinations, callus was induced on the MS media with 0.4–0.5 mg L^{-1} 2,4-D or 0.2 mg L^{-1} TDZ from leaves and petioles, but no adventitious shoots formed from these explants, nor was rooting or transplantation achieved (Su *et al.* 2013).

There are thus no successful reports of shoot regeneration and proliferation of *C. saxicola*. To address this, tissue culture protocols were established for this plant *via* efficient shoot proliferation and regeneration systems allowing plantlets to be mass produced.

Materials and Methods

Plant Material: Collection, Disinfection, and Explant Preparation Several *C. saxicola* mother plants were collected from the wild limestone mountainous area of Bama, Guangxi province, China, in March 2018. They were cultivated in pots (15 cm tall, 15 cm diameter) in a peat substrate. Pots were placed in a greenhouse of the South China Botanical Garden and thoroughly watered every 2 d. Young leaves were excised from mother plants and transported immediately to the laboratory where they were washed with tap water for 30 min. After being transferred to an ultra-clean workbench, explants were disinfected by wiping them with

75% alcohol using cotton balls and cutting them into 5-cm-long explants that were soaked in 0.1% HgCl$_2$ solution for 12 min. Leaf explants were rinsed with sterile distilled water five times, and then trimmed to 1 cm^2 size explants that were inoculated on MS medium supplemented with 2.0 μM of individual PGRs (2,4-D, NAA, BA, and TDZ) (Sigma-Aldrich, St. Louis, MO) for initial tests.

In Vitro **Culture Conditions** Each treatment included 10 culture jars (12 cm tall, 10 cm diameter), each containing five explants. Culture jars were placed in a 25 ± 2 °C culture room in which each shelf was illuminated by two cool white fluorescent lamps (40 W each; Philips, Tianjin, China) at a photon flux density of 80 μmol m^{-2} s^{-1} and a 12-h photoperiod. All media (pH 6.0) contained 3% sucrose and 0.7% agar (Solarbio, Beijing, China). TDZ was filtered (0.22 μm; Corning Filters, Tewksbury, MA) before it was added to autoclave-sterilized medium. Other PGRs were added directly to media prior to sterilization in an autoclave at 121 °C and 104 k Pa for 18 min. After culture for 60 d, adventitious shoot buds that were induced from callus clusters on MS medium with 2.0 μM BA were divided into small clusters (1 cm long) and transferred to the same medium for subculture and proliferation of callus clusters (with adventitious shoot buds). Callus clusters were used in the following tests.

Effect of Medium pH on Adventitious Shoot Induction Callus clusters with adventitious shoot buds that had formed on MS medium with 2.0 μM BA were inoculated on the same medium, but medium pH was adjusted to 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5 (10 culture jars per treatment, five callus clusters per culture jar). After culture for 60 d, the proliferation of callus with adventitious shoot buds and shoot growth was observed while the number of adventitious shoot buds that formed was quantified.

Table 1. Influence of medium pH on callus and shoot proliferation coefficient (SPC) of *Corydalis saxicola* Bunting on Murashige and Skoog medium with 2.0 μM 6-benzyladenine after 60 d

pH	SPC	Observations of organogenesis
4.5	2.8 ± 0.2 e	Leaves light green, callus browning
5.0	3.5 ± 0.3 d	Leaves light green, callus browning
5.5	5.4 ± 0.4 c	Leaves green, callus was hyperhydric
6.0	6.7 ± 0.4 b	Robust shoots, tender green leaves
6.5	7.7 ± 0.4 a	Robust shoots, green leaves
7.0	6.6 ± 0.4 b	Stunted shoots, dark-green leaves, hyperhydricity
7.5	5.6 ± 0.4 c	Stunted shoots, dark-green leaves, hyperhydricity

Each treatment included 50 callus clusters that were cultured in 10 culture jars; each jar contained 5 callus clusters; $n = 50$ per treatment. Different *lowercase letters* indicate statistically significant differences ($P \leq 0.05$; Duncan's multiple range test) between different pH values

Effect of PGRs on Induction of Adventitious Shoot Buds from Leaf and Petiole Explants One-centimeter-long petioles and 1 cm^2 leaves from young *in vitro* shoots were inoculated on MS medium containing different concentrations of PGRs (10 culture jars per treatment, five leaf or petiole explants per culture jar; Table 3). These shoots were derived from callus induced on MS medium with 2.0 μM BA. Based on the outcome of the medium pH tests, medium pH was adjusted to 6.5. After culture for 60 d, callus induction and adventitious shoot formation were recorded. Callus induction percentage
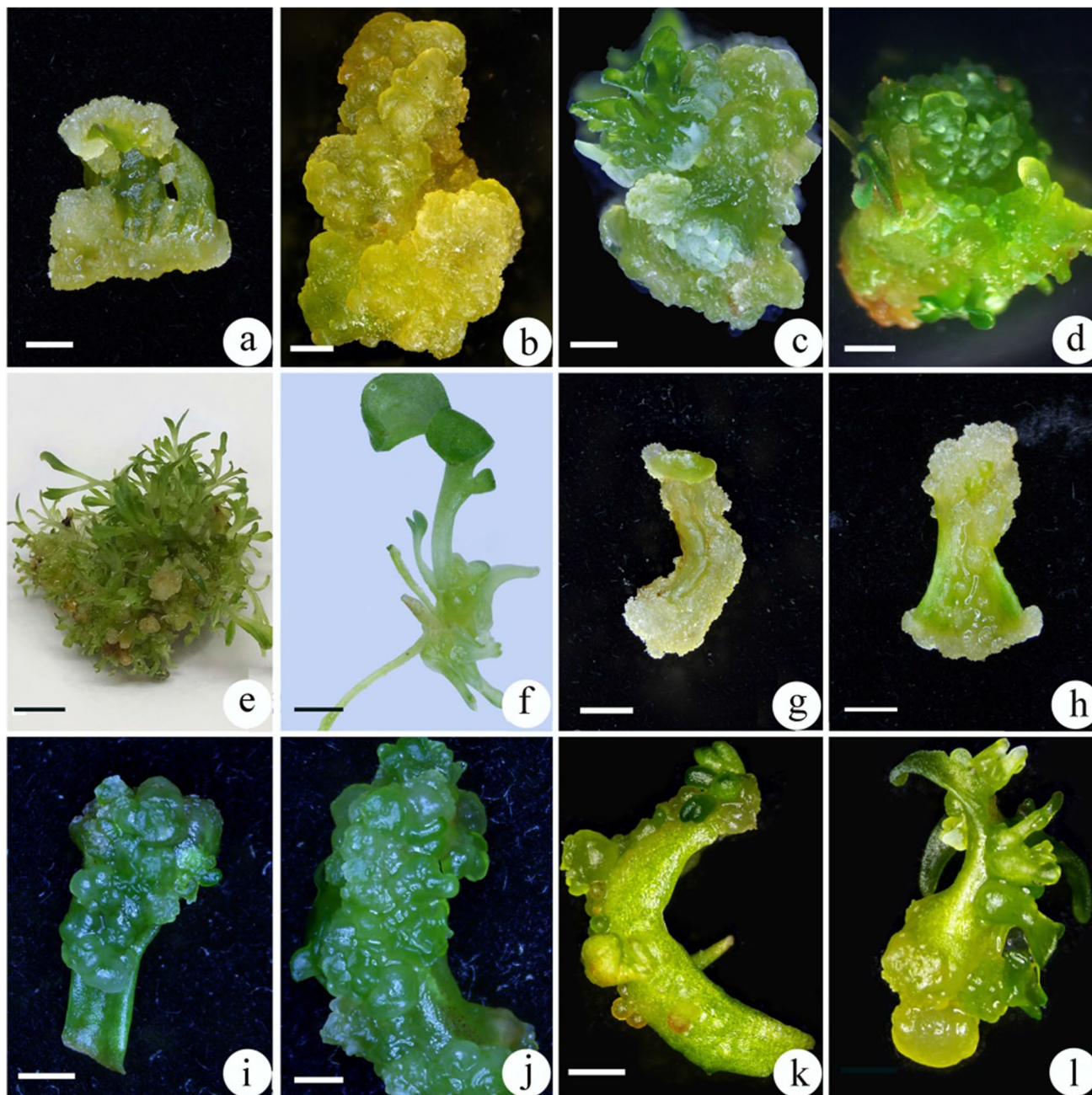

Figure 1. Shoot organogenesis from immature leaf (*a–f*) and petiole (*g–l*) explants of *Corydalis saxicola* Bunting on Murashige and Skoog medium with 2.0 μM 6-benzyladenine and 0.5 μM α-naphthaleneacetic acid. (*a*) Friable callus induced after 20 d. (*b*) Friable callus induced after 30 d. (*c*) Friable callus induced after 40 d showing the formation of some adventitious shoot buds. (*d*) Leaf formation on adventitious shoot buds after 50 d. (*e*) A single callus cluster with multiple adventitious shoot buds after 60 d. (*f*) Following dissection, each shoot formed multiple shoots after 80 d. (*g*) Friable callus was induced after 20 d. (*h*) Friable callus had proliferated after 30 d. (*i, j*) Several protuberances (shoot bud initials) were visible among friable callus after 40 d. (*k*) Adventitious shoots buds were induced after 50 d. (*l*) Adventitious shoots were induced after 60 d. *Bars* = 0.5 cm.

was calculated as follows: (Mean number of leaf / Petiole explants that induced callus divided by total number of leaf or petiole explants) × 100%.

Propagation of Adventitious Shoot Buds Callus clusters, which contained adventitious buds, were cultured on optimal MS medium with 2.0 μM BA. Callus clusters were divided into smaller clusters (1 cm²) and transferred to different MS-based media (pH 6.5) (10 culture jars per treatment, five callus clusters per culture jar). Shoot proliferation coefficient (SPC), for example, the ratio of new shoots formed relative to the original number of shoots, was calculated after 30 d.

Root Induction from Adventitious Shoots Adventitious shoots that were left in MS medium with 2.0 μM BA and 0.5 μM NAA for 60 d were about 3 to 4 cm tall and had 2 to 3 leaves. Individual shoots with leaves were inoculated into ½MS-based medium with different concentrations of IBA (0.1, 0.5, 1.0, and 1.5 μM) and NAA (0.1, 0.5, 1.0, and 1.5 μM), or a mixture of both auxins. Auxin-free MS medium was used as the control group. The pH of all media was 6.5. Five shoots were inoculated per culture jar with 30 culture jars per treatment. After culture for 30 d, rooting percentage was calculated as follows: (Mean number of rooted shoots divided by total number of shoots) × 100%.

Transplantation A total of 60 rooted plantlets with consistent growth status (approximately 6 cm tall), which had formed on ½MS medium with 1.0 μM IBA and 0.2 μM NAA after 30 d, were transplanted into one of two substrates: river sand:vermiculite (1:1, v/v) or peat:vermiculite (1:1, v/v). A total of 30 plants from each substrate were transplanted into plastic pots (10 cm tall, 10 cm diameter). After 90 d in a greenhouse (20 to 30 °C, natural light; light intensity $< 150 \, \mu mol \, m^{-2} \, s^{-1}$), survival percentage was calculated as follows: (Mean number of surviving plants divided by total number of plants) × 100%.

Statistical Analysis Each treatment contained 50 explants. Data are reported as the mean ± standard deviation (SD). Means were statistically analyzed by one-way analysis of variance (ANOVA). Means were considered to be significant between different treatments using Duncan's multiple range test ($P \leq 0.05$) as assessed by SPSS v. 19.0 (IBM, New York, NY).

Results

Effect of Different PGRs on Callus Induction from Leaf Explants On MS medium with 2.0 μM 2,4-D, leaf explants initially induced callus, which eventually browned and died.

Table 2. Shoot organogenesis from *in vitro* leaf and petiole explants of *Corydalis saxicola* Bunting after 60 d

PGRs in MS medium (μM)	Mean number of adventitious shoots per explant	
	Leaf	Petiole
PGR-free	0 fA	0 fA
BA 0.5	2.3 ± 0.1 eA	1.3 ± 0.1 eB
BA 1.0	3.5 ± 0.3 dA	2.4 ± 0.2 dB
BA 2.0	5.8 ± 0.4 bA	4.9 ± 0.2 bA
BA 4.0	5.6 ± 0.4 bA	4.6 ± 0.3 bB
TDZ 0.5	2.2 ± 0.1 eA	2.1 ± 0.2 dA
TDZ 1.0	3.4 ± 0.1 dA	2.2 ± 0.2 dA
TDZ 2.0	4.6 ± 0.2 cA	2.3 ± 0.2 dB
TDZ 4.0	4.5 ± 0.2 cA	3.4 ± 0.3 cA
BA 2.0 and NAA 0.5	7.8 ± 0.4 aA	6.1 ± 0.4 aB
BA 2.0 and IAA 0.5	7.1 ± 0.4 aA	5.6 ± 0.4 aB
BA 2.0 and IBA 0.5	7.4 ± 0.4 aA	6.6 ± 0.4 aB

Each treatment included 40 explants that were divided over 8 culture jars (5 explants per culture jar). Experiments were repeated three times. Different *lowercase letters* indicate statistically significant differences within the same explants (in a *column*) and different *capital letters* indicate statistically significant differences within the same PGR (in a *row*) ($P \leq 0.05$; Duncan's multiple range test). *MS*, Murashige and Skoog; *BA*, 6-benzyladenine; *TDZ*, thidiazuron; *IBA*, indole-3-butyric acid; *NAA*, α-naphthaleneacetic acid

On MS medium with 2.0 μM NAA, little callus was induced, and some adventitious roots formed. On MS medium with 2.0 μM BA, callus was induced at first, after which 5.6 ± 0.3 adventitious shoot buds developed. However, on MS medium with 2.0 μM TDZ, callus was induced, and it then differentiated into 4.7 ± 0.2 adventitious shoot buds.

Effect of pH on Shoot Proliferation and Growth At low pH (4.5 to 5.5), adventitious shoot proliferation coefficient

Table 3. Effects of PGRs on adventitious shoot proliferation coefficient (SPC) of *Corydalis saxicola* Bunting on MS medium after culturing for 30 d

PGRs in MS medium (μM)	SPC
BA 2.0	4.5 ± 0.2 c
TDZ 2.0	4.3 ± 0.3 c
TDZ 2.0 and IBA 0.5	5.1 ± 0.3 b
TDZ 2.0 and NAA 0.5	5.2 ± 0.3 b
BA 2.0 and NAA 0.5	5.8 ± 0.4 a
BA 2.0 and IBA 0.5	5.7 ± 0.4 a

Each treatment included 50 callus clusters that were divided over 10 culture jars (3 callus clusters per culture jar). Different *lowercase letters* indicate statistically significant differences ($P \leq 0.05$; Duncan's multiple range test) between treatments. *MS*, Murashige and Skoog; *BA*, 6-benzyladenine; *TDZ*, thidiazuron; *IBA*, indole-3-butyric acid; *NAA*, α-naphthaleneacetic acid

(SPC) was low, and some callus developed, but leaves on adventitious shoots were hyperhydric. Medium pH of 6.0 to 6.5 was more favorable, SPC was significantly higher than at lower pHs, callus was yellow-green, and leaves were not hyperhydric. At a high pH (7.0), SPC decreased, callus browned, and adventitious buds were thick and hyperhydric. Based on the induced number of adventitious buds and the visual perception of adventitious shoot bud growth, this study determined the most suitable pH to be 6.5 (Table 1).

Shoot Organogenesis from Leaf and Petiole Explants Adventitious shoot buds could be induced from young leaf and petiole explants (Fig. 1). Initially, callus was induced on the

leaf surface within 20 to 30 d (Fig. 1a, b). Adventitious shoot buds then developed from callus; after breaking the surface after 30 to 40 d (Fig. 1c, d), adventitious shoots developed by 50 to 60 d (Fig. 1e, f). The number of adventitious shoot buds increased as BA concentration increased although an excessively high concentration of BA (2.0 to 4.0 μM) induced hyperhydricity of adventitious shoot buds, subsequently reducing their number (Table 2).

Compared with leaves, petioles were more likely to induce callus within 20 to 30 d (Fig. 1g, h), but relatively fewer adventitious shoot buds differentiated within 40 to 60 d (Fig. 1i–l). As cytokinin concentration was increased, the number of adventitious buds that were induced also tended
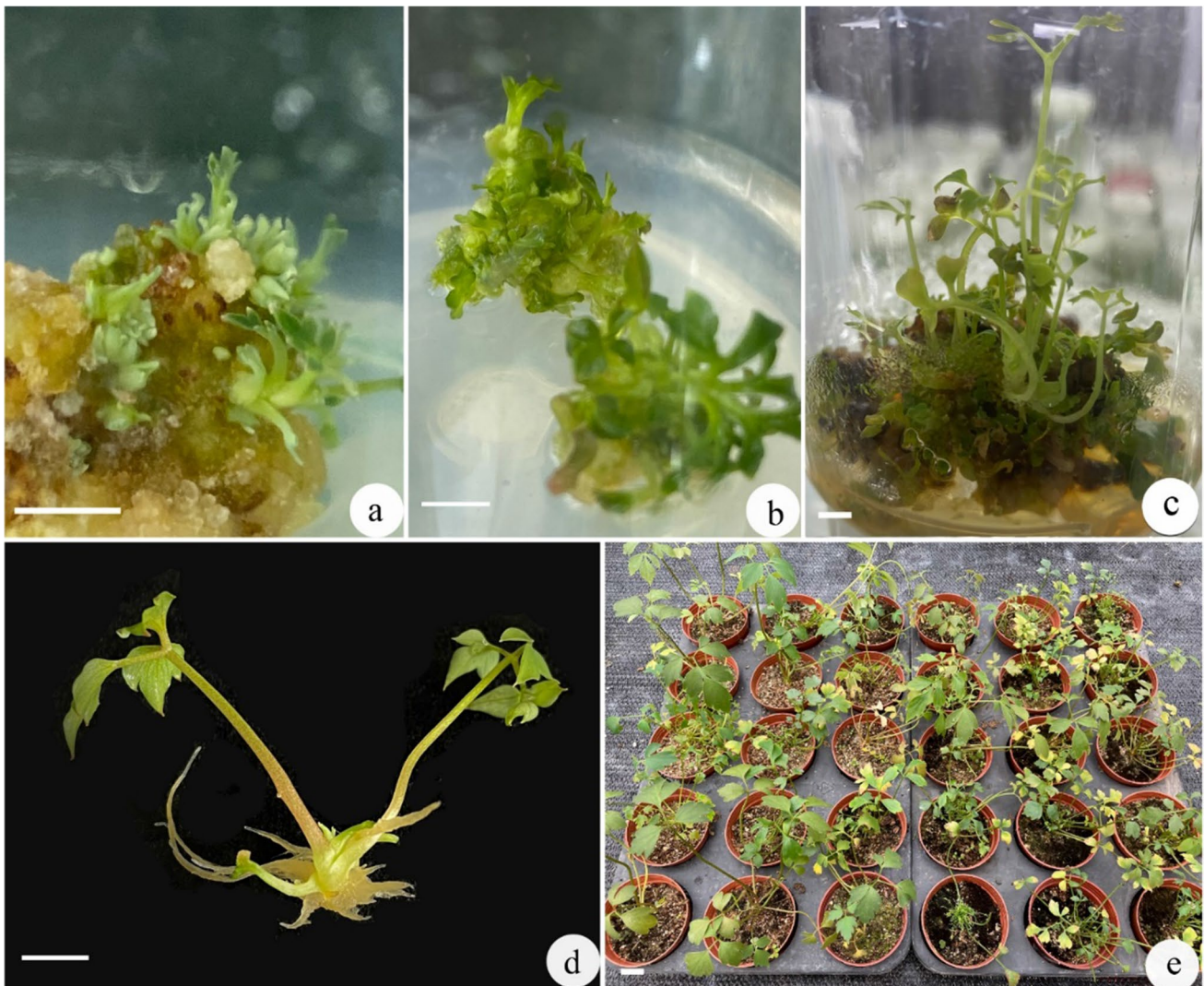

Figure 2. Shoot proliferation and plant regeneration in *Corydalis saxicola* Bunting. (*a*) Callus with adventitious shoot buds was proliferated on Murashige and Skoog (MS) medium with 2.0 μM BA after 30 d. (*b*) Callus with adventitious shoot buds was proliferated on MS medium with 2.0 μM BA and 0.5 μM NAA after 30 d. (*c*) Shoots elongated on MS medium with 2.0 μM BA and 0.5 μM NAA after 60 d. (*d*) After individual shoots were subcultured to ½MS medium with 1.0 μM IBA and 0.2 μM NAA, they rooted, forming plantlets, after 30 d. (*e*) Plantlets were transplanted to two substrates (left tray = river sand:vermiculite; right tray = peat:vermiculite) and photographed after 180 d. BA, 6-benzyladenine; IBA, indole-3-butyric acid; NAA, α-naphthaleneacetic acid.

to increase (Table 3) although callus was also induced, and shoots displayed hyperhydricity. When either 2.0 μM BA or 2.0 μM TDZ was mixed with a low concentration (0.5 μM) of IBA or NAA, the number of adventitious buds that were induced was significantly higher than when either cytokinin was used alone. Leaves induce significantly more callus and adventitious shoot buds than petioles, and most adventitious buds were induced on MS medium with 2.0 μM BA and 0.5 μM NAA (Table 2).

Proliferation of Adventitious Shoot Clusters Callus clusters with adventitious shoot buds were proliferated on MS medium with BA or TDZ (Fig. 2a). When BA (or TDZ) was mixed with a low concentration (0.5 μM) of NAA or IBA, SPC increased (Fig. 2b). MS medium with 2.0 μM BA and 0.5 μM NAA resulted in significantly higher SPC after 30 d (Table 3). When culture period was prolonged to 60 d, several of the larger adventitious shoot buds elongated forming adventitious shoots that were 3 to 4 cm long (Fig. 2c).

Adventitious Root Induction and Transplantation Individual shoots exposed to both NAA and IBA successfully induced adventitious roots (Table 4). However, in the case of NAA, weak adventitious roots formed, and these tended to be scarce and stunted. Moreover, callus was induced at the base of shoots at higher concentrations (2 to 4 μM) with occasional browning, so NAA is not suitable for root induction of *C. saxicola*. In contrast, IBA induced structurally better roots (longer and more abundant) than NAA (Fig. 2d). Ideally, a mixture of both auxins (1.0 μM IBA and 0.2 μM NAA) resulted in significantly higher rooting percentage (95.7%) and root number (7.8 adventitious roots per shoot) after 30 d (Table 4). When *C. saxicola* plantlets were transplanted to river sand:vermiculite or peat:vermiculite substrates, survival after 90 d was 76.67% and 91.25%, while plant was 8.19 cm and 13.79 cm, respectively (Fig. 2e).

Discussion

The objective of this study was to establish an *in vitro* culture system to proliferate *C. saxicola* so that it could be used for applied purposes, thereby reducing anthropomorphic pressure on natural populations, which are geographically limited and thus scarce. Ultimately, while shoot organogenesis was possible from both leaf and petiole explants *via* an indirect callus-mediated stage, the most effective explant was *in vitro* leaves. This was also shown to be effective explants for the micropropagation of other rare or medicinal plants, such as *Metabriggsia ovalifolia* (Ma *et al*. 2011), *Scutellaria bornmuelleri* (Gharari *et al*. 2021), and *Heliotropium foertherianum* (Yu *et al*. 2022).

There have been tissue culture studies of other *Corydalis* species. Somatic embryos of *Corydalis yanhusuo* W. T. Wang were induced from mature tuber-derived callus on agar-gelled MS medium containing 4.56 μM zeatin; as they were converted, somatic embryos were cultured on ½MS medium with 6% sucrose with 0.5 to 10.0 mg L^{-1} abscisic acid, paclobutrazol, or ancymidol; 0.5 to 5.0 mg L^{-1} gibberellic acid (GA$_3$); and 15 to 100 mg L^{-1} polyethylene glycol 4000 for further culture; they developed plantlets and formed *in vitro* tuber (Sagare *et al*. 2001) . Plants with well-developed tubers were cultured on ½MS medium with 2% sucrose and 0.1 mg L^{-1} GA$_3$ for 3 wk, and 80% of somatic embryo–derived plantlets survived in a sand:peat moss substrate after 2 mo (Sagare *et al*. 2001) . Somatic embryos induced using the Sagare *et al*. (2001) protocol developed into plantlets on MS medium with 7.6 μM abscisic acid (Kuo *et al*. 2002). Separately, somatic embryos formed microtubers after being proliferated on a half-strength Linsmaier and Skoog medium with 2% sucrose and 0.1 μM IAA or IBA solidified with 0.2% gelrite germinated on PGR-free White's medium with 2% sucrose and 0.8% agar in the dark at 0 to 4 °C for 180 d (Hiraoka *et al*. 2001). The globular structures observed for *C. saxicola* in this current study were likely adventitious shoots and not somatic embryos (Fig. 2e)

Table 4. Adventitious root induction of *Corydalis saxicola* Bunting on ½ Murashige and Skoog (MS) medium with different auxins after 60 d

½MS medium with auxins (μM)	Rooting (%)*	Number of roots
PGR-free	5.6 ± 0.1 i	2.1 ± 0.2 f
IAA 0.1	12.1 ± 0.2 h	1.4 ± 0.2 e
IAA 0.5	13.7 ± 0.3 h	2.4 ± 0.3 d
IAA 1.0	22.3 ± 0.4 g	2.6 ± 0.4 d
IAA 2.0	34.7 ± 0.4 f	3.6 ± 0.4 c
NAA 0.1	14.4 ± 2.3 h	1.7 ± 0.3 e
NAA 0.5	63.3 ± 2.3 c	2.9 ± 0.4 d
NAA 1.0	66.7 ± 3.3 c	3.7 ± 0.3 c
NAA 2.0	44.4 ± 3.3 e	3.1 ± 0.4 cd
IBA 0.1	12.7 ± 2.6 h	2.3 ± 0.5 d
IBA 0.5	46.4 ± 4.5 e	5.5 ± 1.0 b
IBA 1.0	54.8 ± 3.8 d	4.1 ± 0.8 c
IBA 2.0	45.0 ± 4.7 e	2.5 ± 0.5 d
IBA 1.0 and NAA 0.2	95.7 ± 3.3 a	7.8 ± 0.5 a
IBA 0.5 and NAA 0.5	89.7 ± 2.4 b	6.9 ± 0.5 a
NAA 1.0 and IAA 0.5	87.4 ± 2.5 b	7.4 ± 0.5 a

*Percentage of shoots forming adventitious roots. Each treatment included 50 shoots that were divided over 10 culture jars (5 shoots per culture jar). Different *lowercase letters* indicate statistically significant differences ($P \leq 0.05$; Duncan's multiple range test) between treatments in each *column*. *IAA*, indole-3-acetic acid; *IBA*, indole-3-butyric acid; *NAA*, α-naphthaleneacetic acid

although detailed histological analyses in the future need to clarify this. In *Corydalis bungeana*, callus was induced primarily on MS medium with 0.9 μM BA, 0.4 μM IBA, and 4 to 5 μM NAA; shoots differentiated from callus on MS medium with 3.7 μM BA and 0.5 μM NAA while optimal rooting medium was ½MS medium with 1.0 μM IAA (Tang *et al*. 2011). After plantlets were transplanted directly into pots with river sand and kept at > 90% humidity for 12 d after transplanting, survival was 93.4% after 40 d (Tang *et al*. 2011).

C. saxicola generally grows in limestone areas with alkaline soil (Li *et al*. 2010). This may explain its preferred pH of 6.5 *in vitro* as opposed to lower pHs (Table 2). This study noted that many plant tissue culture studies tend to have a medium pH of 5.8 to 6.0, even some halophytes, such as *Lepturus repens* (G. Forst.) R. Br. (Xiong *et al*. 2021) and *Heliotropium foertherianum* Diane & Hilger (Yu *et al*. 2022).

This study recognized that for plant material to be used in medicine, clonal and genetically identical material is ideal. Future experiments would need to further optimize protocols by identifying specific lines that produce important desired secondary metabolites or medicinally important compounds in high concentrations, refining the current protocols to the production of standard plant material, and perhaps supporting by genetic analyses, such as the use of molecular markers, to ensure the genetic stability and confirm the clonal nature of micropropagated material.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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