MICROPROPAGATION OF *SCUTELLARIA BAICALENSIS* GEORGI

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ABSTRACT

Plant propagation via nodal explants of *Scutellaria baicalensis* Georgi (Lamiaceae) was studied. Murashige and Skoog (MS) medium supplemented either with NAA and kinetin or NAA and BA in different concentrations was used for this purpose. The most favourable for axillary branching was MS medium supplemented with 0.5 μM NAA and 2.5 μM kinetin. The number of regenerated shoots per explant reached 3.8 ± 1.3 . To increase the multiplication rate a two stage procedure of propagation was also elaborated. First, an induction of organogenic calli was achieved using thidiazuron (TDZ) as a sole growth regulator in the nutrient medium (concentration range: 0.1-0.5 μM). Next, regeneration of shoots on the medium containing 2.2 μM BA was performed. Over 20 shoots per explant could be obtained following the described procedure. The regenerated shoots rooted easily on a modified MS medium (1/2 macronutrients) and were successfully adapted to growth in pots.

KEY WORDS: *Scutellaria baicalensis*, axillary shoots, caulogenic callus, micropropagation.

INTRODUCTION

Scutellaria baicalensis Georgi (Lamiaceae), a medicinal plant widely used in the Far East for the treatment of various inflammatory diseases, tumours and arteriosclerosis (Yun-Choi 1992) is a rich source of flavonoids of which baicalin (baicalein-7-O-glucuronide) and wogonin-7-O-glucuronide are the predominant constituents. Some of the flavonoids isolated from *S. baicalensis* proved to be pharmacologically active. They showed an affinity for the benzodiazepine binding site (Liao et al. 1998), bradykinin antagonistic effect (Yun-Choi et al. 1992), antiviral (Konoshima et al. 1992, Nagai et al. 1995), cytotoxic (Ryu et al. 1985), antioxidant (Gabrielska et al. 1997), antiarteriosclerotic and antithrombotic (Kimura et al. 1997a, b, c) activity.

Although some studies concerning cell suspension, callus and root culture of the plant have been published (Yamamoto 1991, Seo et al. 1993, Nishikawa and Ishimaru 1997), to our knowledge, an effective system for in vitro propagation of *S. baicalensis* has not been elaborated yet.

MATERIAL AND METHODS

Plant material

Aseptic plants of *Scutellaria baicalensis* Georgi were obtained from seeds delivered by the Central Garden of Medicinal Plants, Masarykian University (Brno, Czech Republic). The seeds were rinsed with ethanol, sterilized with a 15% solution of sodium hypochlorite and aseptically germinated on a

hormone-free, solidified MS (Murashige and Skoog 1962) medium with 1/2 strength macronutrients, at 25°C and continuous light (ca. 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$, cool white fluorescent tubes). After three weeks, the seedlings were transferred to solidified MS medium and cultivated under the same conditions. Shoots of the aseptic seedlings, ca. 8 cm long, with 5-7 nodes, were used as a source of primary nodal explants.

Culture media

The used nutrient media were solidified with 0.8% Difco Bacto-Agar, contained 3% sucrose as a carbon source and their pH was adjusted to 5.8, before autoclaving (20 min at 121°C).

Axillary shoot regeneration

The nodal explants of *S. baicalensis* were inoculated on solidified MS media supplemented with various concentrations of growth regulators. Two series of media were prepared, containing either α -naphthaleneacetic acid (NAA) and kinetin at a concentration range 0-10 μM (Table 1) or a combination of NAA and 6-benzylaminopurine (BA) (0-5 μM), (Table 2). An effect of 0.1-10.0 μM 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea (TDZ) on shoot formation from node explants of *S. baicalensis* was also studied (Table 3). All cultures were kept at 25°C, under continuous light (ca. 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$, cool white fluorescent tubes).

For each treatment ten explants were used and every experiment was repeated. The multiplication rate, expressed as a number of shoots regenerated from one nodal explant within a culture period and frequency of response, i.e. percentage of

TABLE 1. Shoot formation in nodal explants of *S. baicalensis* inoculated on MS medium supplemented with NAA and kinetin (after six weeks of culture).

NAA [μ M]	Kinetin [μ M]	% response	Number of shoots per explant (> 1 cm)	Roots	Callus *
0	0	35	1.8 ± 0.4	—	—
0	2.5	70	3.6 ± 1.6	—	—
0	10.0	55	4.0 ± 1.9	—	+
0.5	0.5	55	3.8 ± 1.8	—	—
0.5	2.5	70	3.8 ± 1.3	—	—
0.5	5.0	70	2.6 ± 0.5	—	+
0.5	10.0	75	2.8 ± 1.7	—	+
2.5	0	80	1.9 ± 0.3	—	—
2.5	0.5	65	2.1 ± 0.6	+	—
2.5	2.5	90	1.3 ± 0.7	—	+
2.5	5.0	85	2.7 ± 1.5	—	+
2.5	10.0	90	—	—	++
5.0	0.5	75	2.6 ± 0.8	—	+
5.0	2.5	75	2.2 ± 0.9	—	+
5.0	5.0	90	2.0 ± 0.9	—	+
5.0	10.0	100	—	—	++
10.0	0.5	35	0.5 ± 0.2	—	—
10.0	2.5	100	1.9 ± 0.8	—	+
10.0	5.0	100	—	—	+
10.0	10.0	100	—	—	++

* + < 1 g FW

++ 1-4 g FW

TABLE 2. Effect of BA and NAA concentration in the MS medium on shoot formation from node explants of *S. baicalensis* (after six weeks of culture).

NAA [μ M]	BA [μ M]	% response	Number of shoots per explant (> 1 cm)	Roots	Callus *
0	0	40	1.9 ± 0.3	—	—
0	0.5	85	1.4 ± 0.8	—	+
0	2.5	35	—	—	+
0	5.0	30	2.3 ± 1.0	—	++
0.5	0	45	1.7 ± 0.5	—	—
0.5	0.5	35	2.0 ± 0.1	+	+
0.5	2.5	50	5.0 ± 0.8	+	++
0.5	5.0	35	8.5 ± 3.5	—	+
2.5	0	15	—	—	+
2.5	0.5	45	4.0 ± 0.8	—	+
2.5	2.5	20	3.0 ± 1.2	—	+
2.5	5.0	15	—	—	+
5.0	0	35	2.5 ± 0.5	+	—
5.0	0.5	20	4.0 ± 1.1	+	+
5.0	2.5	55	6.8 ± 2.9	—	++
5.0	5.0	35	5.5 ± 1.9	—	+++

* + < 1 g FW

++ 1-4 g FW

+++ > 4 g FW

explants showing either callus or organ formation, were calculated for each treatment.

Rooting of regenerated shoots

After six weeks culture, the regenerated shoots were transferred to rooting medium, i.e. MS medium containing 1/2 macronutrients, without growth regulators. Roots were successfully regenerated within two weeks. The obtained plants were either adapted to growth in pots or were used as a source of secondary explants for the next multiplication step.

Induction of shoot regeneration from caulogenic calli obtained on TDZ containing media

Nodal explants inoculated on MS media supplemented with various concentrations of TDZ produced calli. The calli induced on the media containing 0.1-0.5 μ M TDZ developed numerous shoot buds, however only those cultivated on the medium with 0.1 μ M TDZ were capable to regenerate some shoots (Table 3). To induce shoot development the caulogenic calli, after four weeks of growth on the medium containing 0.5 μ M TDZ, were transferred onto the MS medium sup-

TABLE 3. Effect of TDZ on shoot formation from node explants of *S. baicalensis* (after four weeks of culture).

TDZ [μ M]	% response	Number of shoots per explant (> 1 cm)	Roots	Callus *
0.1	100	2.1 ± 1.0	—	+
0.5	100	shoot buds	—	++
1.0	100	—	—	++
5.0	100	—	—	+++
10.0	100	—	—	+++

* + < 1 g FW

++ 1-4 g FW

+++ > 4 g FW

plemented either with 2.2 μ M BA or with 0.01 μ M BA and 26 μ M of gibberelic acid (GA_3). The shoots obtained within four weeks of growth on those media were rooted following the formerly described procedure.

RESULTS AND DISCUSSION

The most favourable for axillary shoot proliferation in nodal explants of *S.baicalensis* was the MS medium supplemented with 0.5 μ M NAA and 2.5 μ M kinetin (Fig. 1),

though the number of regenerated shoots per explant remained low (3.8 ± 1.3 with 70% frequency). Other medium compositions, especially those containing NAA together with BA induced shoot proliferation with higher multiplication rate (up to 8.5 ± 3.5), but with lower frequency (35-55%). Additionally, regeneration of shoots on those media was accompanied by callus outgrowth. The excised shoots, at least 1 cm long, were individually transferred to rooting medium. During the following two weeks of culture, elongation of shoots (both due to an increase in the length of internodes and the number of nodes) accompanied by root formation was taking place. An average of 4-5 nodes per one regenerated plant was obtained. Rooted plantlets were successfully adapted to the growth in pots (Fig. 2).

Several studies were devoted to micropropagation of *Lamiaceae* species through proliferation of axillary shoots. Culture media supplemented either with sole cytokinin (preferably BA) or with a combination of cytokinin with low concentration of auxin were used for this purpose. Tavares et al. (1996) obtained 24 axillary shoots per cotyledonary node explant of *Melissa officinalis* L. after 60 days culture on MS medium containing 8.9 μ M BA. Optimum shoot organogenesis through adventitious bud proliferation from apical meristem explants was achieved in *Thymus vulgaris* L. (Shetty et al. 1996) and *Origanum vulgare* L. (Yang and Shetty 1998) on MS medium supplemented with 4.4 μ M BA. Pattnaik and

Fig. 1. Axillary shoot development from node explant of *Scutellaria baicalensis* Georgi on MS medium supplemented with 0.5 μ M NAA and 2.5 μ M kinetin.

Fig. 2. In vitro regenerated plant of *Scutellaria baicalensis* Georgi adapted to growth in a pot.

Chand (1996) elaborated a method of in vitro propagation of *Ocimum americanum* L. and *Ocimum sanctum* L. using axillary shoot buds. They found that the optimum BA concentration for proliferation of shoots was 1.1 μM for *O. americanum* and 4.4 μM for *O. sanctum*. According to their study an addition of GA_3 to the medium improved both frequency of bud break and multiplication rate. Olszowska and Furmanowa (1992) used a combination of adenine sulfate (10 mg/l) and 3-indolebutyric acid (IBA) (0.5 mg/l) for clonal propagation of *Thymus vulgaris* L. from stem nodal segments. Sáez et al. (1994) applied MS medium supplemented either with 6.6 μM BA and 2.8 μM indole 3-acetic acid (IAA) or with 4.4 μM BA and 1.0 μM NAA to induce shoot regeneration from shoot explants of *Thymus piperella* L. In the course of the present study it was shown that BA at a concentration range of 0.5–5.0 μM , preferably used in the mentioned studies, had no stimulatory effect on a multiple shoot regeneration from single node explants of *S. baicalensis*. Some combinations of BA with NAA (e.g. 2.5 μM BA with 0.5 μM NAA and 2.5 μM BA with 5.0 μM NAA) stimulated bud break and improved multiplication rate with reference to the control (Table 2). The shoot regeneration however, was accompanied by callus formation.

To achieve a higher multiplication rate from stem nodal explants of *S. baicalensis*, a two stage propagation system was elaborated using thidiazuron, which is one of the most potent synthetic cytokinins and is widely applied to induce in vitro organogenesis in woody plants (Huetteman and Preece 1993). In the first stage, nodal explants of *S. baicalensis* were inoculated on MS medium containing 0.5 μM TDZ. After four weeks culture, a caulogenic callus emerged from each explant. The ratio of the final weight of the callus to the weight of explant reached 318 ± 95 . The calli were transferred to the MS medium containing either 0.01 μM BA and 26 μM GA_3 or 2.2 μM BA (second stage). Multiplication rates achieved with those media, after the following four weeks, were 20 ± 9.8 and 25 ± 6.8 shoots per node, respectively. The excised shoots, at least 1 cm long, were further cultivated on modified MS medium (1/2 macronutrients) and 80% of shoots formed roots within two weeks. Although the two stage procedure of *S. baicalensis* micropropagation was shown to be efficient, a risk of somaclonal variation caused by indirect shoot regeneration should be taken into consideration.

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MIKROROZMNAŻANIE *SCUTELLARIA BAICALENSIS* GEORGI

STRESZCZENIE

Optymalizowano warunki dla rozmnażania in vitro roślin *Scutellaria baicalensis* Georgi (tarczycza bajkałska) (Lamiaceae) z fragmentów pędów zawierających węzeł i parę liści. Zastosowano pożywkę Murashige-Skoog'a (MS), do której dodawano NAA i kinetynę lub NAA i BA w różnych stężeniach. Najkorzystniejsza dla bezpośredniej regeneracji pędów z pączków pachwinowych była pożywka zawierająca 0,5 μ M NAA i 2,5 μ M kinetyny, pozwalająca na uzyskanie $3,8 \pm 1,3$ pędów z jednego eksplantatu, po sześciu tygodniach hodowli. W celu zwiększenia wydajności procesu mikrorozmnażania, opracowano również dwufazową procedurę regeneracji pędów z pączków pachwinowych. W pierwszej fazie, na pożywce zawierającej TDZ (0,1-0,5 μ M), uzyskiwano organogeny kalus z licznymi pąkami przybyszowymi. W drugiej fazie indukowano regenerację pędów, na pożywce zawierającej 2,2 μ M BA. Procedura ta pozwoliła na uzyskanie ponad 20 pędów z jednego eksplantatu. Zregenerowane pędy ukorzeniały się łatwo na pożywce MS ze zmniejszoną do połowy zawartością makroelementów i były z powodzeniem adaptowane do wzrostu w glebie.

SŁOWA KLUCZOWE: *Scutellaria baicalensis*, pąki pachwinowe, kalus kaulogeny, mikrorozmnażanie.