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# Tissue Culture and Micropropagation of Mongolian Licorice (*Glycyrrhiza uralensis* Fisch.)

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## Abstract

Mongolian licorice (*Glycyrrhiza uralensis* Fisch.) is a pharmacologically important plant rich in flavonoids and saponins. For tissue culture, root and cotyledon explants from seedlings were used. Sterilized explants with one node were used for micropropagation. Half-strength Murasige-Skuge medium and Gamborg's B5 medium with different supplements were used for the induction of calluses and multiple shoots. Conditions for tissue culture and micropropagation of *G. uralensis* were determined.

**Key words:** callus, *Glycyrrhiza uralensis*, *in vitro*, micropropagation, Mongolia, nodal culture

## Introduction

Currently, there is much international interest in increasing plant resources, plant productivity and the ability to synthesize specific compounds, especially various secondary metabolites useful for medicinal practices.

Licorice (*Glycyrrhiza uralensis* Fisch.), belonging to the family *Fabaceae*, is recognised as one of the most valuable and widely used oriental herbs. During the last few years, use of licorice has increased rapidly. However, due to human influence and natural desertification processes in Mongolia licorice resources may become exhausted in the near future.

*In vitro* culture and propagation are useful tools in the conservation of this important plant. Use of *in vitro* cultures of *G. uralensis* and *G. glabra* L. have been reported by a number of authors (Thengane *et al*., 1998; Kovalenko & Kurchii, 1998; Kohjyouma *et al*., 1995). The roots and isolated active components of these plants are widely used for treatment of viral infections, inflammatory diseases and prevention of different cancers (Arase *et al*., 1997; Numazaki, 2003; Shiraki *et al*., 2003).

The aim of our research is to determine methods of *in vitro* culture and micropropagation of Mongolian licorice, in order to increase the bioresources of *G. uralensis* in Mongolia.

## Materials and Methods

**Plant materials.** Seed samples of *G. uralensis* were provided by researchers from the Institute of Botany at the Mongolian Academy of Sciences. Two and four year old *G. uralensis* leaves, roots and whole plants were also collected from Dashinchilen district in Bulgan province, and Bogd and Baatsagaan districts in Bayankhongor province during a field study.

**Culture conditions.** The seeds were soaked in sterilized water for 24 hours, followed by a 70% ethanol bath for 30 seconds. Stratifications were performed at 4$^{o}$C for 72 hours, and the seed surface sterilized with 2% sodium hypochloride solution with a drop of Tween-20 for 5 minutes, before being rinsed with sterile water 3 times. The prepared seeds were then used for germination and microprogation experiments.

For germination of *G. uralensis*, half-strength basal Murashige-Skoog medium (MS medium; pH 5.8) supplemented with 0.8 % agar and 1.5 % sucrose was used. Autoclaving was performed at +121 $^{0}$C for 15 minutes (Oyunbileg & Mijidsuren, 2004).

Seedlings grown on Gamborg's B5 medium and supplemented with 4 different combinations of growth regulators (BAP-1.0 mg/L and 2.0 mg/L, IAA-0.1 mg/L and 1.0 mg/L) (Liao *et al*., 2004) were used for micropropagation.

**Incubation.** All cultures were incubated at 25 $^0$C under 8/16 hours photoperiod in a daylight cabinet. Light intensity was 40 µMole·m$^{-2}$ · s$^{-1}$.

## Results and Discussion

Naturally grown licorice has poor seed germination, about 1 % to 8 % (Gankhuyag & Ligaa, 1993; Dashjamts, 1983). Gankhuyag and Ligaa (1993) stated that it is possible to achieve 92 % seed germination through scarification, and previous researchers also determined that seed germination could be increased after mechanical damage to the seed coat (Gankhuyag & Ligaa, 1993; Dashjamts, 1983). In this study, seed coats were damaged and the stratification method was used, as a result 55.8 % - 100 % of seeds germinated. There was no significant difference between mediums (1/2 MS, 1/2 B5, 1 % agar) used in the experiment as shown in Figure 1.

One week after germination, root and cotyledon explants from seedlings were transferred to Gamborg's B5 medium and supplemented with 1 mg/l 2.4-dichlorphenoxyacetic acid (2.4-D), 0.1 mg/l kinetin and 2% sucrose to initiate callus growth. Two to three weeks later, callus initiation occurred (Table 1).

Calluses that grew from root explants were more friable, yellowish in colour and watery compared with those initiated from cotyledon explants (Fig. 2).

Propagation of *G. uralensis* was performed using nodal and shoot tip cultures, on Gamborg's B5 medium, which was supplemented with 5 different combinations of BAP and IAA growth regulators. There was no significant difference between the lengths of shoots and the addition of BAP and IAA combinations did not increase shoot formation (Table 2).

In addition, the seedlings were grown on half-strength MS medium supplemented with 4 different combinations of BAP and NAA growth regulators. The results showed that half-strength MS medium with 0.1 mg/l NAA was effective in inducing the root of licorice (Table 3). After 14 days of culture, the medium with no growth regulators was effective in the formation of multiple shoots and roots. Some shoots rooted spontaneously on half-strength MS medium without growth regulators. This medium was therefore selected for rooting and regeneration of whole plantlets. As a result, after 42 days of culture the plantlets developed (Fig. 3), but only 50 plantlets were transplanted into pots. Currently we are continuing our research, and regenerated plantlets are being acclimatized.

The nodal cultivated on MS medium formed new shoots after 10 days. In order to identify the optimal medium for regeneration, cultures from

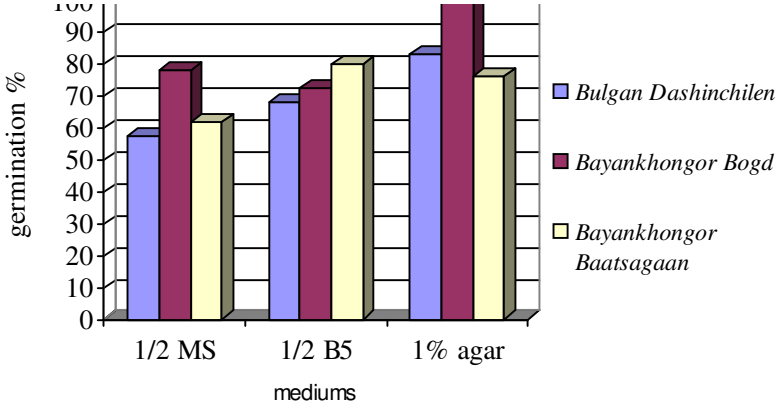


Figure 1. Comparative seed germination of *G. uralensis* (in 1/2 MS, 1/2 B5, 1 % agar mediums) after one week, collected from 3 different locations.

Table 1. Callus initiation from root and cotyledon explants, after 3 weeks.

| *Location of sample* | *Explant* | *Number of tested explants* | *Number of induced calluses* |
|---|---|---|---|
| Bulgan: | Cotyledon | 30 | 25 |
| Dashinchilen | Root | 30 | 27 |
| Bayankhongor: | Cotyledon | 30 | 26 |
| Baatsagaan | Root | 30 | 28 |
| Bayankhongor: Bogd | Cotyledon | 30 | 25 |
| | Root | 30 | 28 |

Table 2. Effect of BAP and IAA on root formation and shoot development

| Treatments | Concentration of growth regulators (mg/l) | | Explants tested (N) | Root formation rate (%) | Length of shoot (cm) |
|---|---|---|---|---|---|
| | BAP | IAA | | | |
| 1 | 0 | 0 | 9 | 55.6 | 2.72 ± 0.18 |
| 2 | 2.0 | 0.1 | 9 | 0 | 2.50 ± 0.25 |
| 3 | 2.0 | 1.0 | 9 | 0 | 2.66 ± 0.15 |
| 4 | 1.0 | 0.1 | 9 | 0 | 2.33 ± 0.27 |
| 5 | 1.0 | 1.0 | 9 | 0 | 2.61 ± 0.24 |

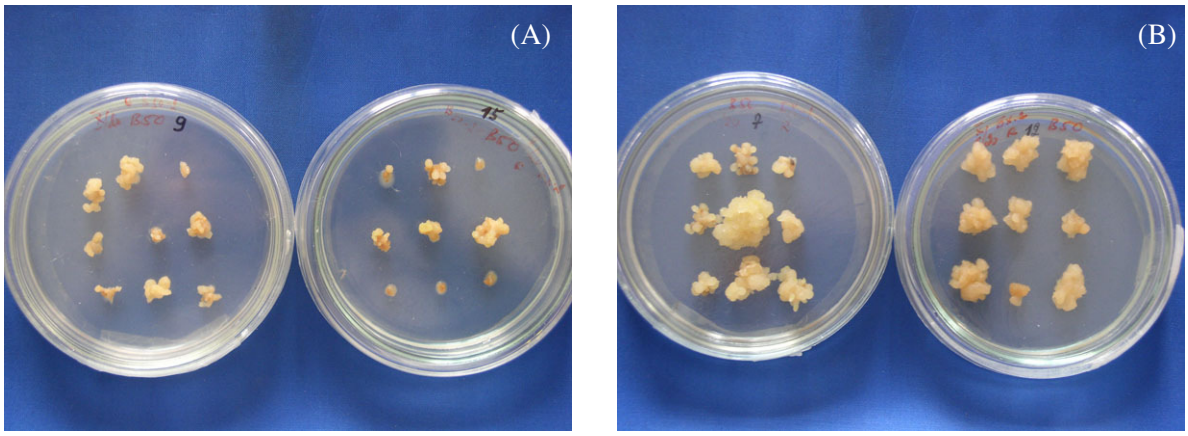


Figure 2. Callus grown from the cotyledon (a.) and the root (b.) explants.

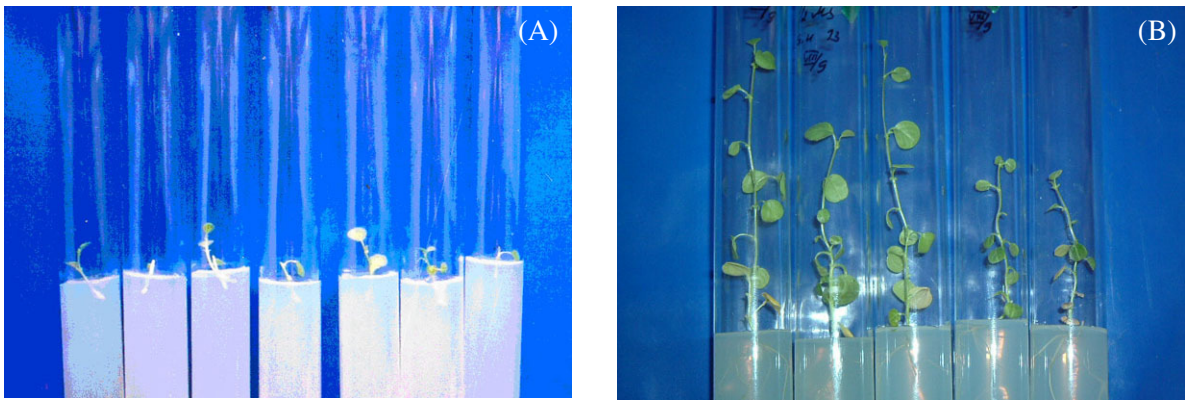


Figure 3. A. Transferred shoot tips of *G. uralensis* into new 1/2 MS medium. B. After 6 weeks.

Figure 4. New shoot formation by nodal cultures and influence of secreted phenolics.

seedlings and multiple shoots from nodal cultures were sub-cultured on half-strength MS medium. The results of our experiments showed that regenerated plantlets could be obtained after about 3 weeks (Fig. 4). These results were similar to those conducted by Thengane *et al.* (1998) and Kohjyouma *et al.* (1995) who developed *in vitro* plantlets of *Glycyrrhiza glabra*.

One problem raised during the *in vitro* micropropogation was the secretion of phenol-like substances, which inhibited the growth of tissue culture. The same phenomenon was observed by Kovalenko *et al.* (1998). In this case, the secretion of phenolics was regulated by abscisic acid, which acts as an antioxidant. In our experiment, it was found that frequently transferring the new shoots

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| 4 | 1.0 | 0.1 | 9 | 0 | 2.33 ± 0.27 |
| 5 | 1.0 | 1.0 | 9 | 0 | 2.61 ± 0.24 |

to new medium prevented the phenol-like substances from influencing culture growth.

The results obtained from this study would be useful for increasing *G. uralensis* resources and for secondary metabolism research.

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## Хураангуй

Урал чихэр өвс (*Glycyrrhiza uralensis* Fisch.) нь флавоноид болон сапонины төрлийн нэгдлүүдээр баялаг, чухал ач холбогдолтой эмийн ургамлын нэг юм. Урал чихэр өвсний эдийн өсгөврийг хийхэд in vitro-д үрийг соёолуулсан цухуйцаас үрийн тал, үндэсний эксплант, бичил үржүүлэг хийх зорилгоор ариутгасан үе зайдмыг хэрэглэв. Чихэр өвсний каллус, нахиаг хөгжүүлэхэд ялгаатай гормоны хувилбартай Гамбург B5, Мурашиге-Скугийн хагас найрлагатай тэжээлийн орчин хэрэглэсэн юм. Лабораторийн нөхцөлд зарим бичил ургамаланцар, эсийн биомасс гаргаж авах боломжтой болох нь бидний судалгааны зарим үр дүнгээс харагдаж байна.

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