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In Vitro Propagation from Axillary Buds of *Glycyrrhiza glabra* L.

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Stem segments with axillary buds of *Glycyrrhiza glabra* L. were cultured on modified MS medium supplemented with α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). Formation of multiple shoots was obtained on the medium provided with 1.00 mg/*l* BAP after 40 days of culture.

Each shoot was cut and transplanted onto the medium supplemented with NAA and BAP. After 80 days of culture, the rooting of shoots was well established in the media containing 0.01-0.50 mg/*l* NAA and these shoots subsequently developed into whole plants.

Introduction

In the Pharmacopeia of Japan Twelfth Edition, Glycyrrhizae Radix, licorice, is the root and stolon with or without periderm of *Glycyrrhiza uralensis* Fisher, *G. glabra* L. or other species of the same genus. Licorice are derived from at least five *Glycyrrhiza* species. Licorice has been used as a sweetener as well as an important drug in oriental medicine. In Japan, all products derived from licorice are imported. Most licorice is harvested from wild plants, so the quality of the products are not stable enough to use in drugs. Therefore, demand for cultivation of *Glycyrrhiza* plants which are used as licorice, has increased recently. Commonly these plants are propagated by stolon division but this conventional method is slow and vulnerable to viral infection during propagation.

The object of this study is to establish a rapid *in vitro* propagation method. In this paper, we reported the formation of multiple shoots and subsequent plant regeneration.

Materials and Methods

1. *Preparation of explants*

The subterranean stems of *Glycyrrhiza glabra* L. plant, cultivated at Kumano Medicinal Plants Garden of Saraya Co., Ltd. were cut into the 15-20 cm lengths. They were washed with tap water for 1-2hr and soaked in 5% calcium hypochlorite solution for 10 min. They were transplanted to vermiculite, and cultivated in a growth chamber at 25±2°C in continuous light of fluorescent lamps (about 3,000 lux). After one month of cultivation, the 15-20 cm shoots growing in the pots were divided into 5 cm long segments with a few axillary buds. They were washed with tap water for 1 hr, surface sterilized with 1% sodium hypochlorite solution with a drop of Tween 20 (1 drop/50 m*l*)

for 5 min., and then rinsed with sterile water 3 times. They were cut into 1 cm long segments each containing one axillary bud using sterilized scissors.

2. *Effect of NAA and BAP on shoot formation*

The sterilized segments were cultured in 16 kinds of modified Murashige and Skoog media[1] which organic components were changed to one of Gamborg B5 medium[2], each containing both NAA at concentrations 0, 0.04, 0.20, 1.00 mg/*l* and BAP at concentrations 0, 0.2, 1.0, 5.0 mg/*l*. All basal media were provided with 3% sucrose and solidified with 0.8% agar. They were statically cultured in bottles (180 m*l*) containing about 30 m*l* medium adjusted to pH 5.5 before autoclaving. The culture bottles were covered with alminium sheets and exposed to light for 16 hr (3,000 lux at 25±2°C).

3. *Effect of BAP on induction of multiple shoots*

Furthermore to test the effect of BAP on shoot formation, various concentrations of BAP (0.25, 0.50, 1.00, 2.00 mg/*l*) were added to the basal medium.

4. *Induction of root*

After 40 days of primary culture, the newly-formed multiple shoots were cut into individual shoots, and transferred onto fresh rooting media supplemented with various combinations of NAA (0.05, 0.10, 0.50 mg/*l*) and BAP (0, 0.01 mg/*l*). When the shoots were cultured in the rooting media for 40 days, they were transferred onto the same type of fresh media (50 m*l*) solidified with 0.6% agar in Erlenmeyer flasks (500 m*l*).

Light and temperature conditions for the culture were the same as those given above.

Results and Discussion

1. *Formation of multiple shoots*

After 40 days of culture, morphogenetic changes were observed. The media containing BAP without NAA were effective in the formation of multiple shoots. These shoots were abnormally shaped compared to the mother plant. Calli formation was mainly observed on the media provided with 5.0 mg/*l* BAP with 0.04-0.20 mg/*l* NAA or without NAA (**Table 1**). So we regarded high concetration of BAP such as 5 mg/*l* as tending to induce calli formation in this plant.

In the next experiment, the effect of BAP on multiple shoot formation was investigated. 50 days after culture initiation, the number of shoots (more than 1 cm in length) per explant was recorded for each culture (**Table 2**). The highest number of shoots, 4.6 per explant, was obtained on the medium with 1.00 mg/*l* BAP (**Fig. 1-A, B**). Therefore this concentration of BAP was selected as a best condition for the formation of multiple shoots for use in the following experiment.

2. *Root formation and plant regeneration*

When the newly formed shoots were transferred to the rooting media for 80 days, root initiation and shoot elongation were observed (**Table 3**). The highest frequency of root formation was obtained on the medium with 0.05-0.10 mg/*l* NAA. In this condition, development of the shoots and length and number of leaves were as in a normal plants (**Fig. 1-C**). The addition of BAP did not increase root formation.

The plantlets developed on the rooting medium were rinsed with water and transplanted into pots with vermiculite and covered with a plastic film to maintain high humidity. There after, the plantlets were grown in a greenhouse (**Fig. 1-D**). It is possible to obtain over 150 seedlings a year using the tissue culture technique, compared with 4 or 5 seedlings a year by conventional stolon cuttings.

Single shoot regeneration directly from the axillary bud of *Glycyrrhiza glabra* L. has been

Table 1. Effect of growth regulators on morphogenesis of axillary buds culture.

Concentration of growth regulators		Number of tested explants	Callus*1/Root*2	Shoot formation rate(%)	Number of shoot per explant
NAA	BAP (mg/*l*)				
0	0	15	0/3	80.0*3	2.4±0.5*3,*4
0	0.2	15	1/0	26.7	2.5±1.0
0	1.0	15	1/0	86.7	3.4±1.5
0	5.0	15	7/0	6.7	3.0±0.0
0.04	0	15	0/7	60.0	1.2±0.7
0.04	0.2	15	0/0	33.3	1.4±0.9
0.04	1.0	15	4/0	26.7	2.0±1.4
0.04	5.0	15	6/0	0.0	0.0±0.0
0.20	0	15	0/8	80.0	1.1±0.3
0.20	0.2	15	0/0	60.0	2.3±1.1
0.20	1.0	15	1/0	80.0	2.2±1.9
0.20	5.0	15	1/0	0.0	0.0±0.0
1.00	0	15	2/2	73.3	1.0±0.0
1.00	0.2	15	0/0	66.7	1.3±0.7
1.00	1.0	15	0/0	46.7	1.4±1.1
1.00	5.0	15	0/0	20.0	1.0±0.0

*1 Number of explant which formed callus.
*2 Number of explant which formed root.
*3 After 40 days of culture, shoots were counted on the shoots elongated over 5 mm in length.
*4 Number of shoots per explant.
Each value represents mean±standard deviation.

Table 2. Multiple shoots formation from the axillary bud.

Concentration of BAP(mg/*l*)	Number of tested explant	Shoot formation rate(%)	Number of shoots per explant
0.25	15	80.0*1	3.1±1.5*1,*2
0.50	15	73.3	3.0±1.7
1.00	15	73.3	4.6±2.1
2.00	18	80.0	3.8±1.9

*1 After 50 days culture, shoots were counted on the shoots elongated over 1 cm in length.
*2 Number of shoots per explant.
Each value represents mean±standard deviation.

reported[3]. The present paper explains the method of multiple shoot induction and plant regeneration of *G. glabra* L.

The results obtained in this paper will be useful for providing a large number of seedlings and for breeding better strains.

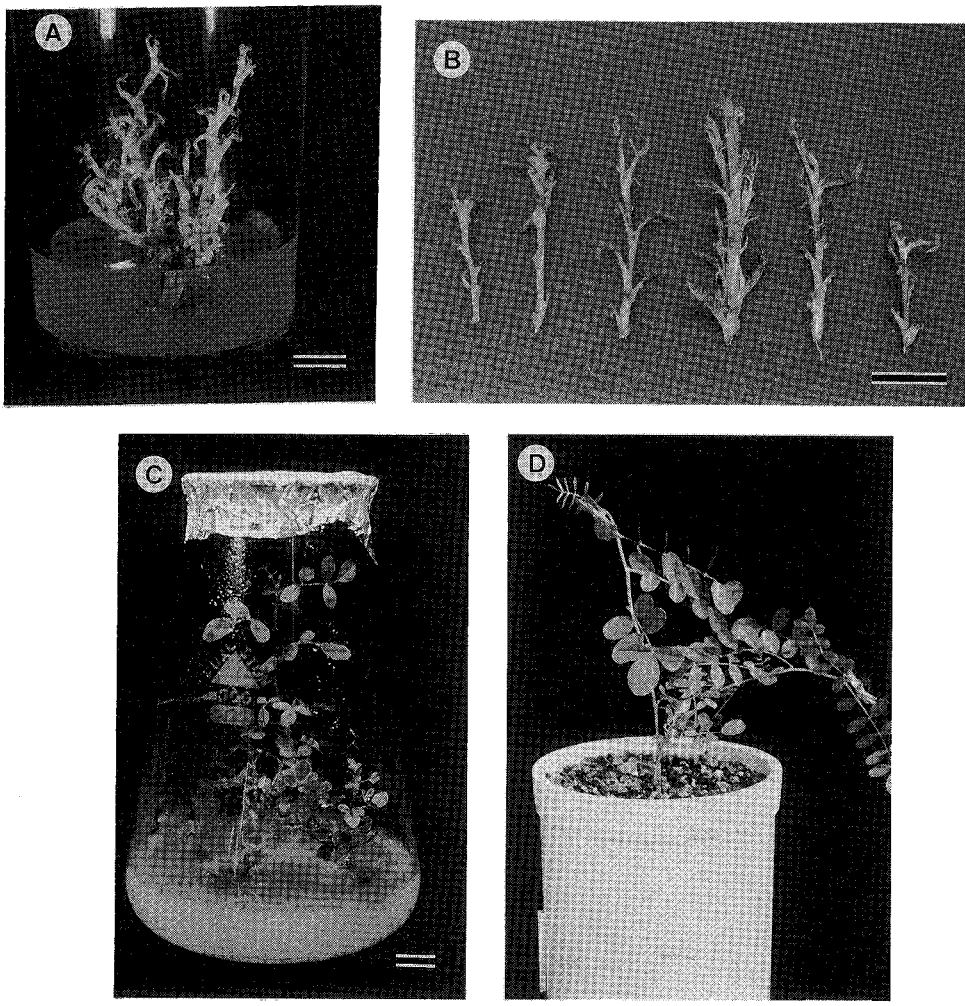

Fig. 1 A: Multiple shoots developed from an axillary bud of *G. glabra* after 50 days of culture.
B: Shoots cut from a multiple shoot.
C: Regenerated shoot with roots.
D: Plantlet obtained after acclimatiztion.
Bars in photographs indicate 1 cm.

Table 3. Root formation and shoot development in the rooting medium from shoots obtained from axillary bud culture.

Concentration of growth regulators		Number of shoots tested	Root formation rate(%)	Length of shoot (cm)	Number of leaves per shoot
NAA	BAP (mg/*l*)				
0.05	0	9	77.8	4.6±1.7*[1]	46.7±23.6*[1]
0.10	0	9	77.8	6.2±4.2	34.2±24.1
0.50	0	9	55.6	4.7±3.8	36.1±32.3
0.05	0.01	9	33.3	3.2±2.8	12.1±14.0
0.10	0.01	9	33.3	3.5±2.6	18.6±17.4
0.50	0.01	9	55.6	4.0±2.7	24.6±28.7

Root formaion and shoot development were observed 80 days after transplanting to the rooting medium. After 40 days of first subculture in the rooting medium, each shoot was inoculated onto fresh medium.
*[1] Each value represents mean±standard deviation.

References

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《和文要約》

カンゾウ(*Glycyrrhiza glabra* L.)の腋芽からの試験管内増殖

高上馬希重*・神田博史*・谷　尚美**・芦田　馨**・杉野　守**・山本章彦***・堀越　司***

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*** サラヤ株式会社

カンゾウ(*Glycyrrhiza glabra* L.)の萌芽後1ヶ月の植物体を用いて試験管内増殖について検討した．改変MS培地にBAP 1.00 mg/*l* のホルモン条件で腋芽からマルチプルシュート形成が得られた．このシュートを切断分離し継代培養を行うと，NAA 0.05-0.10 mg/*l* 添加した培地が発根に最適で，幼植物を再生し健全な植物体が得られた．