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武庫川女子大紀要（自然科学）

Callus Formation from *Eucommia ulmoides* (*Tu-chung*) Branch

Tokumitsu Okamura, Yukiko Inoue and Yasuko Ohfuka

Department of Food Science and Nutrition,
School of Human Environmental Sciences,
Mukogawa Women's University, 6-46, Ikebiraki-cho, Nishinomiya 663-8558, Japan.

The fruits of *Eucommia ulmoides* are used as medical uses-such as to strengthen the intestinal organs and heart. In this paper, we report successful results in the induction of callus from branch of *E. ulmoides*. The medium containing 0.01μM of 2,4-D and 0.1μM of BA effectively induced callus.

Introduction

Eucommia ulmoides Oliver(*Tu-chung*)is one of the oldest herbs known and, from ancient times, its medical uses-such as to strengthen the intestinal organs and heart, to provide vigor of spirit, to remove fatigue, to prevent aging, and to strengthen bone and muscles-have been noted in China[1].

However, its precise metabolic effects were not clear. Several investigators have confirmed the hypotensive action of aqueous and ethanol extracts of *E. ulmoides* bark in various animals[2-4].

In this paper, we report the induction of callus from branches of *E. ulmoides*

Materials and Methods

Induction and culture of callus

A herb garden grown *E. ulmoides* plants of approximately ten years of age were used. Branches of this plant were rinsed in 70% (v/v)ethanol for 30 seconds, sterilized by immersion for 5 minutes in 5% sodium hypochlorite solution containing 0.01 ml/L of Tween 80, and rinsed three times in sterilized distilled water. The sterilized branches were cut into blocks (1cm x 1cm) with a surgical knife and placed on Murashige and Skoog medium (MS medium)[5]supplemented with 3% sucrose, various concentration (0, 0.01, 0.1, 1μM) of 2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphthaleneacetic acid(NAA), indole-3-acetic acid(IAA), 6-benzyladenine(BA), kinetin and zeatin. The solid medium was solidified by 0.2% Gelrite. The culture were incubated in dark at 25℃.

Results and Discussion

Induction and culture of callus

The media containing 0.01μM of 2,4-D and 0.1μM of BA effectively induced callus as shown in Fig. 1. The callus was transparent and friable. The callus on the medium contained 0.01μM of 2,4-D and 0.01μM of kinetin was opaque green and compact in the subculture process (Fig. 2.(a)). Whereas the medium containing 1μM of kinetin caused browing of callus in the subculture process(Fig. 2.(b)). And also the white callus was obtained with the liquid culture medium containing more than 0.01μM of 2,4-D after 20 days of incubation (Fig. 3.). Fig. 4. shows the microphotograph of the white callus.

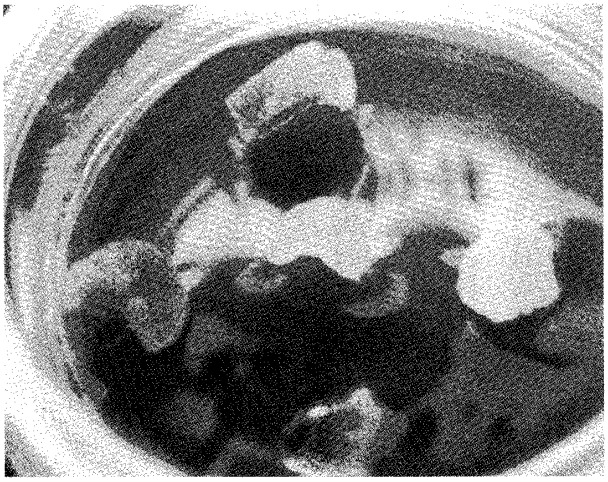

Fig. 1. Callus formation from a peeled branch of *E. ulmoides*.

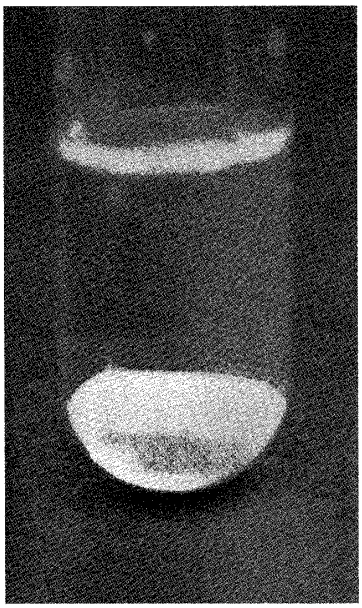

Fig. 3. White callus formation from a peeled branch of *E. ulmoides* with liquid culture medium contained 0.01μM of 2,4-D and 0.01μM of kinetin.

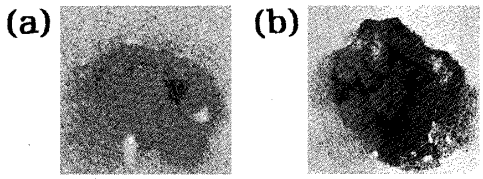

Fig. 2. Subcultured callus
(a) Opaque green and compact callus (the medium contained 0.01μM of 2,4-D and 0.01μM of kinetin).
(b) Opaque brown and compact callus (the medium contained 1μM of kinetin).

Fig. 4. Microphotograph of the white callus.

Table 1. Effects of concentration of NAA, IAA, 2, 4-D, BA, kinetin and zeatin on callus formation in *E. ulmoides* branch.

	Callus formation (%)											
	BA (μM)				Kinetin (μM)				Zeatin (μM)			
	0	0.01	0.1	1	0	0.01	0.1	1	0	0.01	0.1	1
NAA (μM)												
0	0	0	0	0	0	0	0	0	0	0	0	0
0.01	0	0	0	0	0	0	0	0	0	100	100	100
0.1	0	0	0	0	0	100	100	100	0	100	100	100
IAA (μM)												
0	0	0	0	50	0	0	0	0	0	0	0	0
0.01	0	100	100	100	0	0	0	0	0	0	0	0
0.1	0	100	100	100	0	0	0	100	0	0	100	100
2, 4-D (μM)												
0	0	0	0	50	0	0	0	50				
0.01	0	100	100	100	0	100	100	100				
0.1	0	100	100	100	0	100	100	100				

Callus was observed after 20 days culture.

Table 1. shows the frequency of callus formation from branch explants after 20 days culture. In particular, in the case of 0.1μM of NAA, the better results were obtained with the medium containing kinetin and zeatin, and in the case of 0.01μM or 0.1μM of 2,4-D, the better results were obtained with the medium containing BA or kinetin. On the other hand, the better results were obtained with the medium containing 0.01μM or 0.1μM of IAA and more than 0.01μM of BA.

Acknowledgments

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References

1) Chen, T.J., Commercial Press, Shanghai, pp.66(1934)
2) Chin, K.C. and Ting, K.S., *Acta Physiol. Sin.*, **20**, 247(1956)
3) Chien, T.H., *Jpn. J. Pharmacol.*, **3**, 122(1957)
4) Chan, B.Y.F., Cheng, K.K. and Li, K.M., *Far East Med. J.*, **6**, 259(1970)
5) Murashige, T. and Skoog, F., *Physiol. Plant.*, **15**, 473-497(1962)