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A Medicinal plant, *Eucommia ulmoides*: Possibility of In Vitro Propagation under Several Tissue Culture Conditions

Manami Inoue, Hiroko Nakatsuka, Arisa Noguchi, Wakanori Amaki and [1]Chieko Yasuma

Graduate School of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa 243-0034, Japan: [1]Hekizanen Limited Company, 1438-5 Hanbara, Aikawa, Aiko, Kanagawa 243-0307, Japan

43322003@nodai.ac.jp

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Summary

Eucommia ulmoides Oliver (eucommia) is a deciduous and dioecious woody plant that is native to China. In China, the bark has been used as a Chinese herbal medicine since ancient times, and its medicinal properties include lowering blood pressure, analgesia, and diuresis. Recently, it has been discovered that the leaves have similar effects. There have been several reports of propagation by tissue culture in the past using in vitro seedlings, but it cannot be said to be established. In the future it will be necessary to conduct line selection and propagation of eucommia. For this purpose, the following experiments were conducted using axillary buds of adult trees. We added 6-benzylaminopurine (BA) and 2,3,5-triiodobenzoic acid (TIBA) in combination and investigated their effects. As a result, by combining 2 mg/l BA and 1 mg/l TIBA and increasing the culture temperature to 28°C, adventitious shoots could be induced.

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INTRODUCTION

Eucommia ulmoides Oliver (eucommia) is a deciduous woody plant that is native to China and belongs to the family Eucommiaceae, order Eucommiales. *Eucommia* belongs to one family, one genus, and one species, and is dioecious. In China, the bark has been used as a Chinese herbal medicine since ancient times, and its medicinal properties include lowering blood pressure, analgesia, and diuresis. Recently, it has been discovered that the leaves have similar effects (Harashima, 2012). There is currently a lot of fallow land in Aikawa Town, Aiko District, Kanagawa Prefecture, and local companies are using the fallow land to cultivate eucommia. At the same time, they are already producing and selling eucommia tea using eucommia leaves. The city has begun to revitalize through the sixth industrialization. However, production of eucommia leaves, the raw material for the product, has not kept up with demand.

Currently, seeds are mainly used to propagate eucommia, but only female trees set seeds, and the germination rate of seeds is extremely low when using normal sowing methods. There have been several reports of propagation by tissue culture in the past (Chen et al., 1995; Chen et al., 2007; 2008), but it cannot be said to be established. Furthermore, previous tissue culture propagation studies have prepared explants from hypocotyls of sterile seedlings. When we look at the field-grown eucommia, they are genetically mixed, perhaps because they are propagated by seedlings. In the future it will be necessary to conduct line selection and propagation of eucommia. For this purpose, it is necessary to induce multiple shoots from explants prepared from adult trees. The following experiments were conducted using axillary buds of adult trees.

MATERIALS AND METHODS

Seedlings provided by Hekizanen were planted, and branches were collected from a 6-year-old adult trees grown at the Atsugi campus of Tokyo University of Agriculture. The axillary buds prepared from collected branches were used. We conducted some preliminary experiments and found that the contamination rate was extremely high when using general sterilization methods. Therefore, we investigated various sterilization methods to reduce the contamination rate, and finally decided to sterilize the axially buds using the following method. The collected axillary buds were dipped in a solution of 1.25 g/L of benomyl (GF Benlate hydrating agent, Sumitomo Chemical Garden Products Inc.) and 1 drop of Tween 20, and then treated with an ultrasonic cleaner (ASU-6, As One Co., Ltd.) for 10 minutes. The axillary buds were sterilized by immersion in 70% ethanol for 5 minutes and then 2% sodium hypochlorite (NaClO) containing one drop of Tween 20 for 10 minutes in a clean bench. Thereafter, the axially buds were washed three times with sterilized water, the scale leaves of axially bud were removed with a scalpel, and the center of axillary buds were cut out to form explants.

The basic medium was a 1/2 strength Murashige and Skoog (1962) medium to which 30 g/l sucrose and 8 g/l agar were added, and the pH was adjusted to 5.8. A flat-bottomed glass test tube (φ40 x 130 mm) was used as the culture vessel, and after dispensing 40 ml of the medium, the tube was closed with aluminium foil and

sterilized in an autoclave machine (LSX-500, TOMY SEIKO Co., Ltd) at 120°Cfor 15 minutes.

Primarily, the effects of plant growth regulators on axillary bud development were investigated using 2 mg/L 6-benzylaminopurine (BA), 2 mg/L 1-naphthaleneacetic acid (NAA), and 1 mg/L 2,3,5-triiodobenzoic acid (TIBA). The culture environment was 23 ± 1℃, white fluorescence light 20 μmol m^{-2} s^{-1} photosynthetic photon flux density PPFD, 16 hours light/8 hours dark. Although axillary bud development was observed in this experiment, there was a lot of white friable callus formation, and subsequent bud growth could not be observed. Therefore, we next used an incubator (CFH-305, TOMY SEIKO Co., Ltd.) for culture, changed the culture temperature to 28 ± 4.5℃, and investigated the effects of mixed 2 mg/L BA and 1 mg/L TIBA on shoot formation, which BA had been shown to be effective on axillary bud development in the previous experiment.

RESULTS AND DISCUSSION

There are few research reports on tissue culture of eucommia, and even when we conducted follow-up tests using previously reported methods using in vitro seedlings (Chen et al., 1995; Chen et al., 2007; 2008), there was almost no response. In this experiment, the addition of 2 mg/l BA was found to have some effect on promoting the axillary bud development (**Table 1**) but did not lead to adventitious shoot formation.

Table 1. Effects of TIBA, BA, and NAA on the development of axillary bud explants in *Eucommia ulmoides*.

Plant growth regulator (mg/L)			Survival rate (%)	Contamination rate (%)	Development of axially bud (%)
TIBA	BA	NAA			
0	0	0	65	0	0
1	0	0	60	0	0
0	2	0	75	0	15
0	0	2	20	0	0

*Culture temperature is 23±1°C; 16-hr light (PPFD at 20 μmol·m^{-2}·s^{-1}) / 8-hr dark. n=20)

Although not shown in the data, addition of 1 mg/L TIBA tended to suppress callus formation and proliferation. Therefore, we added BA and TIBA in combination and investigated their effects. As a result, by combining 2 mg/l BA and 1 mg/l TIBA and increasing the culture temperature to 28°C, adventitious shoots could be induced (**Table 2** and **Fig. 1**).

Table 2. Effects of TIBA and BA on the morphogenesis of axillary bud explants in *Eucommia ulmoides*.

Plant growth regulator (mg/L)		Survival rate	Contamination rate	Callus formation	Development of axially bud	Adventitious shoot formation
TIBA	BA	(%)	(%)	(%)	(%)	(%)
0	0	5	25	5	0	0
1	2	5	15	5	5	0
1	2	15	20	5	5	5

*Culture temperature is 23±1°C; 16-hr light (PPFD at 20 μmol·m^{-2}·s^{-1}) / 8-hr dark. n=20)

Figure 1. Adventitious shoot formation of axillary bud explants from adult tree on 1/2MS + 2 mg/L + 1 mg/L TIBA.

The fact that adventitious buds could be induced from the axillary buds of adult trees indicates that eucommia is dioecious, and that it is possible to select and breed superior female plants. The results of a series of experiments on eucommia suggest that the timing of collecting axillary buds, the ripeness of the branches, and the position of the axillary buds on a branch affect the results. Furthermore, the types and concentrations of plant growth regulators used need to be further optimized, and further research is required.

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