Genetic Transformation of *Eucommia ulmoides* Oliv. with the Late Embryogenesis Abundant Protein Gene*

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Eucommia ulmoides Oliv. calli were induced on an MS medium supplemented with 2.0 mg l⁻¹ 2,4-dichlorophenoxy acetic acid (2,4-D), and a good suspension was obtained in a liquid medium after 3 rounds of subculturing. The suspension cells were transformed by particle bombardment using the *pBY520* plasmid containing the late embryogenesis abundant protein gene from barley (*Hordeum vulgare* L.), namely, the *LEA3* gene. After 4 rounds of selection culturing on a medium containing phosphinothricin, 338 resistant calli were obtained.

Key words: *Eucommia ulmoides* Oliv.; *LEA3* gene; transformation

Introduction

Since *Eucommia ulmoides* is a Tertiary species that has survived only in China, it is a living fossil plant. It is the only species in both its genus and family. *E. ulmoides*-derived medicine had been described in “*Shen Nong Ben Cao Jing*,” an ancient Chinese medical book, as sweet, slightly pungent, warm in nature, and non-toxic. Its pharmacological functions are reducing blood pressure, nourishing the liver and kidney, strengthening the muscles and bone, preventing aging, and stimulating protein synthesis in muscle. The bark has been used as an antihypertensive, diuretic, analgesic, sedative in traditional Chinese medicine since ancient times. Modern scientific research has reported that the *E. ulmoides* leaf also has some pharmacological effects that are similar to those of the bark ¹⁻⁵.

Besides bioactive substances, *E. ulmoides* gum (EU gum) is another important component that is produced in the bark, leaves, and pericarp, and it plays an important role in the rubber industry ⁶. The novel materials derived from EU gum cover a wide spectrum of materials—from improved functional plastic elastomers and thermoelastomers to high elastomers. Many novel materials have been made using vulcanized EU gum, e.g., temperature controlled switches, multiple function connectors, sealing materials for stopping leaks, antennae, artificial limb sockets, and protective vests for sportsmen.

Currently, *E. ulmoides* is widely planted in Korea, Japan, North Europe, and North America. Since soil salt stress and drought impose restrictive effects on its production, it is necessary to develop salt- and drought-resistant *E. ulmoides* cultivars.

The gene transformation technique has been widely used in research concerning economic trees, and a number of species of transgenic plants including kiwifruit ⁷, papaya ⁸,

citrus fruit ^{9,10}, apple ^{11,12}, banana ¹³, grape ^{14,15}, and strawberry ¹⁶ have been produced. However, the use of the gene transformation technique in *E. ulmoides* has not been reported.

To increase the resistance of salt stress to *E. ulmoides* and reduce the effects caused by soil salt stress and drought on its production, a late embryogenesis abundant protein gene, *LEA3*, from barley (*Hordeum vulgare* L.) was introduced into the *E. ulmoides* genome in the present study.

Materials and Methods

1. Callus induction and suspension culture

E. ulmoides seeds were collected from trees and sterilized using 0.1% (W/V) HgCl₂ for 7 minutes; this was followed by 4 washes with sterile water. The seeds were then inoculated into a petri dish containing 1/2 MS medium (pH 5.8) under illumination (20 µE m⁻² s⁻¹) at 25 °C. The sterile seedlings were harvested 4 weeks later.

The cotyledons (0.2 × 0.2 cm) and hypocotyls (0.5 cm) of the sterile seedlings were used as explants for callus induction on an MS medium containing 0.6% (W/V) agar and 3% (W/V) sucrose supplemented with 2.0 mg l⁻¹ 2,4-D (pH 5.8) under illumination (20 µE m⁻² s⁻¹) at 25 °C. The induced calli were subcultured 3 times under the same conditions as used for callus induction, and then they were suspended in an MS medium without agar containing 3% (W/V) sucrose, 300 mg l⁻¹ lactalbumin hydrolysate (LH), and 2.0 mg l⁻¹ 2,4-D and shaken in a shaker at a rate of 120 rpm at 25 °C ¹⁷.

2. Structure of the *pBY520* plasmid

The structure of the *pBY520* plasmid is shown in Fig. 1. The *LEA3* gene is promoted by rice *Act1* and terminated by potato *Pin2*. The plasmid also contains the bacterial phosphinothricin acetyl transferase structural gene, commonly

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Fig. 1. Structure of the plasmid *pBY520*

known as the *bar* gene, which serves as a selectable marker and is regulated by the CaMV 35S promoter and the nopaline synthase gene (*nos* gene) termination signal.

3. Gene transformation

E. ulmoides cells from the suspension were used as transformation materials and bombarded with tungsten particles coated with the *pBY520* plasmid using the PDS-1000/He gene gun (Bio-Rad). The bombarded cells were transferred to an MS medium supplemented with 2.0 mg l⁻¹ 6-BA (6-benzylamino-purine), 0.2 mg l⁻¹ indoleacetic acid (IAA), and 10.0 mg l⁻¹ phosphinothricin (PPT) for selection. The medium also contained sucrose (30 g l⁻¹) and agar (7 g l⁻¹), pH 5.8. Subculturing was performed every 10 days. After 4 rounds of selection culturing using PPT, resistant calli were obtained.

Results and Discussion

1. Callus induction and suspension culture

Explants were cultured on an MS medium supplemented with 2.0 mg l⁻¹ 2, 4-D. The induced calli grew rapidly and were very loose, grain-like, and suitable for suspension culturing. A good suspension could be obtained after 3 rounds of subculturing on a liquid medium.

2. Gene transformation and transgenic calli selection

Sixty plates of *E. ulmoides* cells were transformed by particle bombardment using the *pBY520* plasmid containing the *LEA3* gene. The bombarded cells were transferred to an MS medium supplemented with 2.0 mg l⁻¹ 6-BA, 0.2 mg l⁻¹ IAA, and 10.0 mg l⁻¹ PPT for selection. After 4 rounds of selection culturing, 338 resistant calli were obtained. The mean number of resistant calli selected per bombarded plate was 5.6; this was similar to the result (5.4/bombarded plate of strawberry calli) reported by Wang, J.L. *et al.*¹⁷⁾ and the result (200 resistant calli/33 plates of rice cells bombarded or 6.1/plate) reported by Xu, D.P. *et al.*¹⁸⁾ (Table 1).

Resistant *E. ulmoides* calli were obtained by PPT selection, suggesting that the foreign gene had been integrated into the *E. ulmoides* genome. Further experiments such as regeneration of resistant calli and research regarding the salt tolerance and drought resistance of transgenic plants will be performed.

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Table 1. Summary of transformation

Transformation experiment no.	No. of plates of cells bombarded	No. of resistant calli selected
1	20	116
2	20	112
3	20	110
Total	60	338

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