

ノート (Note)

Development of ozone-susceptible transgenic poplars with a sense DNA for 1-aminocyclopropane-1-carboxylate synthase

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エチレン合成酵素遺伝子の過剰発現による オゾン感受性遺伝子組換えポプラの開発

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Ethylene is a plant hormone involved in the regulation of growth and development in response to environmental stresses (Wang et al. 2002). It is synthesized from S-adenosyl-L-methionine via 1-aminocyclopropane-1-carboxylate (ACC) in higher plants, and ACC synthase (ACS; EC 4.4.1.14) often catalyzes the rate-limiting step in ethylene biosynthesis (Yang and Hoffman 1984). Previously, we succeeded in improving the O₃ tolerance of tobacco (Nakajima et al. 2002) and poplar plants (Mohri et al. 2011) using an antisense DNA for the early O₃-inducible *ACS* gene. In the present study, we succeeded generating O₃-susceptible transgenic poplar plants by over-expression of the *ACS* gene. To our knowledge, this is a first report of transgenic woody plants with increased susceptibility to air pollutants.

The transformation and regeneration of Lombardy poplar (*Populus nigra* L. var. *italica* Koehne) were performed as described previously (Mohri et al. 1996, 2011). A blunt-ended cDNA for the O₃-inducible *ACS* gene (PO-ACS2, accession No. AB033503) was ligated in a sense orientation to the cauliflower mosaic virus 35S promoter and replaced the β -glucuronidase coding region of the binary vector pBI121-Hm (Fig. 1). The disarmed *Agrobacterium tumefaciens* strain LBA4404, harboring the binary vector, was used in the transformation experiments.

Transgenic poplar plants were grown weekly with 0.1% (v/v) Hyponex (Hyponex Japan Licensee, Tokyo, Japan), and pots were watered daily. Four- to 5-week-old transgenic plants were exposed to 0.2 and 0.6 ppm O₃ in a growth chamber (230 cm

× 190 cm × 170 cm) at 25°C and 70% relative humidity, under light from metal halide lamps with a photosynthetic photon flux density of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Ozone was generated from an O₃ generator (Sumitomo Seika Chemicals Co., Tokyo, Japan).

Sixteen transgenic lines that showed kanamycin and hygromycin resistance (markers of a successful transformation) were obtained. All transgenic plants contained the introduced hygromycin phosphotransferase gene (data not shown). The morphological features of the transgenic plants did not differ noticeably from the control plants (Fig. 2). A difference in growth rates was not found under strong light and sufficient nutrient conditions; however, a difference was sometimes observed when cultivation occurred under weak light and insufficient nutrient conditions (Fig. 2). We examined the physiological characteristics of transgenic poplar plants to understand their potential for O₃ susceptibility. When the control poplar plants were exposed to 0.2 ppm O₃, no leaf damage was found. When the O₃ concentration increased to 0.6 ppm, the leaves withered and necrosis appeared on the surface within 24 h after the end of exposure (Fig. 3). In contrast, three randomly selected transgenic lines showed visible damage at 0.2 ppm O₃ and one line (No. 9) showed the highest susceptibility to the O₃ treatment (Fig. 3). The injuries tended to be more extensive in older leaves than in younger leaves.

In conclusion, the present study shows that the constitutive expression of a sense DNA for an O₃-inducible *ACS* gene could increase the O₃ susceptibility of poplar plants.

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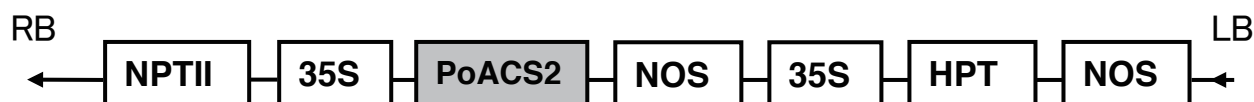


Fig. 1. Structure of the construct used for the transformation of Lombardy poplar. NPT II, a gene encoding neomycin phosphotransferase II; 35S, cauliflower mosaic virus 35S promoter; NOS, nopaline synthase terminator; HPT, a gene encoding hygromycin phosphotransferase; RB/LB, right/left border of T-DNA.

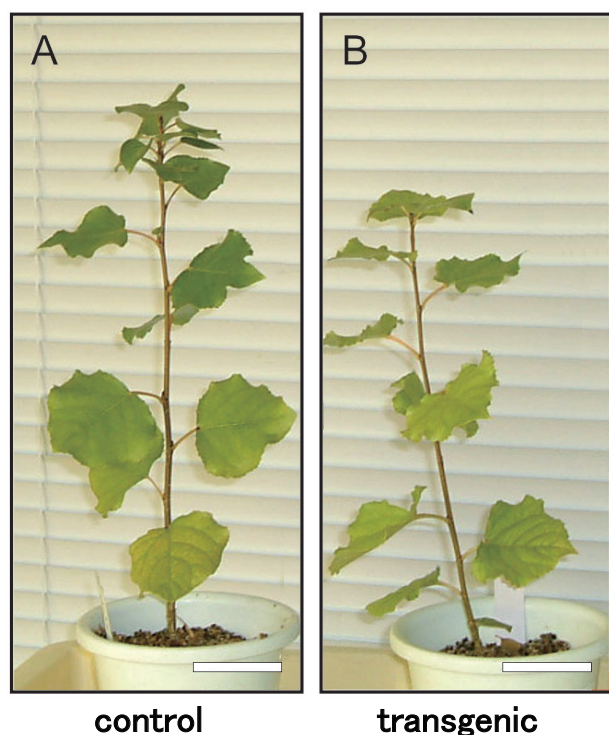


Fig. 2. Morphological features of transgenic Lombardy poplar plants. Plants were grown with 0.001% (v/v) Hyponex, under cool, white fluorescent light ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$, 16 h photoperiod). Bar = 10 cm.

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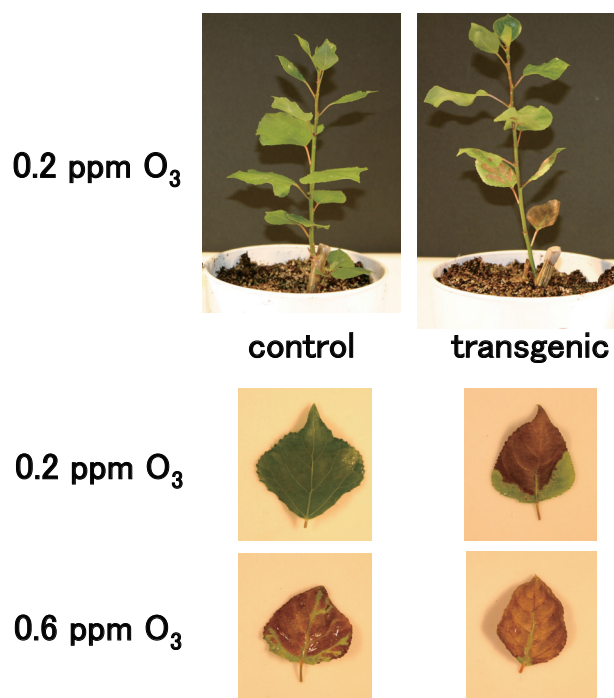


Fig. 3. Ozone susceptibility of transgenic Lombardy poplar plants. Four- to 5-week-old control and transgenic (line 9) poplar plants were exposed to 0.2 and 0.6 ppm O_3 for 6 h. After the O_3 treatment, they were left in the light for 24 h.

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