「森林総合研究所研究報告」(Bulletin of FFPRI) Vol.13 No.3 (No.432) 89 - 98 September 2014

論　文（Original article）

Drought and salt stress tolerance of ozone-tolerant transgenic poplar with an antisense DNA for 1-aminocyclopropane-1-carboxylate synthase

Satoshi KOGAWARA[1], Takeshi MOHRI[2)*], Tomohiro IGASAKI[2)], Nobuyoshi NAKAJIMA[3)] and Kenji SHINOHARA[2)]

Abstract

We examined the drought and salt stress tolerance of transgenic poplar (*Populus nigra* var. *italica* Koehne) with an antisense DNA for 1-aminocyclopropane-1-carboxylate synthase (ACS). Our previous report had already revealed that this transgenic poplar had higher ozone tolerance. Compared with the wild-type, drought-induced senescence and defoliation of mature leaves was ameliorated in the transgenic poplar. Under salt stress, the transgenic poplar showed less foliar injury and maintained a higher photosynthetic activity than the wild-type. The mature leaves showed induction of ethylene biosynthesis under drought and salt stresses, which was attenuated in the transgenic plants as observed under ozone stress. Although the expression of *ACS* gene in the mature leaves was induced by both drought and salt stress, the level of induction was lower in the transgenic poplar than in the wild-type. Therefore, it is assumed that the *ACS* gene is responsible for the increase in ethylene biosynthesis under these stresses. These results suggest that down-regulation of the *ACS* gene by the introduction of an antisense DNA is effective in enhancing the tolerance of poplar to multiple stresses.

Key words : ACC synthase, environmental stresses, Lombardy poplar, suppression of ethylene biosynthesis

1. Introduction

Genetic engineering has the potential to allow selective improvement of individual traits in forest trees without the loss of any of the desired traits of the parental line. Using such techniques, we can overcome the difficulties associated with the breeding of long-lived perennials that need a long time to produce progeny. As a result of environmentally damaging development and unsustainable land use, the expansion of degraded land has recently become a social problem around the world. Afforestation and reforestation of abandoned land is important to restore biological productivity and to improve carbon sequestration. Genetically modified woody plants tolerant to environmental stresses are expected to contribute to the rehabilitation of such degraded land.

Plants are known to increase ethylene biosynthesis in response to environmental stresses. Ethylene 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). Increased ethylene biosynthesis under various stresses has been attributed to induced ACS activity. Ethylene is a plant hormone involved in regulation of growth and development in response to environmental stresses (Wang et al. 2002), and in disease resistance (van Loon et al. 2006), although a high concentration of ethylene is usually deleterious to plant growth (Czarny et al. 2006, Pierik et al. 2006). Increased ethylene production accelerates senescence and often causes foliar injury. For example, ozone, the main oxidant component of photochemical smog is known to induce ethylene production in plant species, which is considered to correlate with the level of leaf necrosis (Nakajima et al. 2002). Genetic engineering to decrease *ACS* expression is expected to efficiently attenuate the deleterious effects of excess ethylene production. Previously, we succeeded in improving the ozone tolerance of tobacco (Nakajima et al. 2002) and poplar plants (*Populus nigra* var. *italica* Koehne; Mohri et al. 2011) with an antisense DNA for the early ozone-inducible *ACS* gene.

The environment in degraded land is often complicated and a variety of abiotic stresses are imposed on plants living there. For a transgenic tree with high tolerance to a particular stress, evaluation of its tolerance to other environmental stresses is important for its applicability to the rehabilitation of degraded land. Since ethylene is known

Received 21 February 2014, Accepted 19 June 2014
1) Asian Natural Environmental Science Center, The University of Tokyo
2) Department of Molecular and Cell Biology, Forestry and Forest Products Research Institute (FFPRI)
3) Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies
* Department of Molecular and Cell Biology, FFPRI, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan; e-mail: mohri@ffpri.affrc.go.jp

to participate in signal transduction in response to various stresses, down-regulation of the *ACS* gene is expected to provide tolerance not only to ozone (Mohri et al. 2011) but also to other environmental stresses. In the present study, we found that ozone-tolerant transgenic poplar also has tolerance to drought and salt stresses. We will discuss the availability of genetic engineering through the suppression of ethylene biosynthesis by a down-regulation of the *ACS* gene.

2. Materials and Methods

2.1 Plant material

The ozone-tolerant transgenic poplar (*Populus nigra* var. *italica* Koehne) used in this study was the same line described previously (Mohri et al. 2011), in which an antisense DNA for the early ozone-inducible *ACS* gene was introduced and over-expressed under the control of the cauliflower mosaic virus 35S promoter, resulting in reduced induction of endogenous *ACS* expression and ethylene production by ozone stress than in wild-type poplar. Young trees of both wild-type and transgenic poplar were obtained by propagation of rooted cuttings. Rooting of these poplars was achieved in 300 mL pots filled with vermiculite that were irrigated twice a week with nutrient solution (Shinohara et al. 1998). They were grown in a room maintained at 25°C, under a photosynthetic photon flux density (PPFD) of 60 μmol photons m^{-2} s^{-1} in a 16h-light/8h-dark cycle. They were transplanted to the experimental system for drought or salt stress treatment when their shoot length reached 10–20 cm.

2.2 Drought stress treatment

Cuttings (approximately 15 cm height) were transplanted into 1.0 L plastic pots filled with washed sand that were irrigated daily with water containing nutrients as described previously (Shinohara et al. 1998). Ten days after transplanting, the plants were transferred to an artificial-light room maintained at 25°C and 70% relative humidity, with a PPFD of 200 μmol photons m^{-2} s^{-1} from metal halide lamps during a 16-h photoperiod, and were allowed to acclimate to the experimental conditions for one month. For half of the cuttings, drought stress treatment was initiated by withholding irrigation and was continued for 5 days. During the treatment, sand moisture was measured by inserting a theta probe (ML2x; Delta-T Devices, Ltd., Cambridge, UK) into the sand. The treatment was applied to three replicates for both wild-type and transgenic plants.

2.3 Salt stress treatment

Cuttings (approximately 15 cm height) were transplanted to hydroponic culture in 20-L plastic tanks containing 15 L of the nutrient solution described above, which was aerated by bubbling air at a rate of 150 mL min^{-1} and was replenished every 5 days. In each tank, a polyvinyl chloride plate with holes to allow the shoots to protrude was floated on the surface of the nutrient solution. Each cutting was suspended from the lid through a hole padded with sponge to seal the space. To acclimate to the hydroponic culture system, cuttings were grown in an artificial-light room with a PPFD of 60 μmol photons m^{-2} s^{-1} for 3 weeks after transplanting and were rotated between tanks. Thereafter, half of the cuttings were placed in nutrient solution with 80 mM NaCl added. This concentration was selected to prevent rapid dehydration resulting from a decrease in water uptake. The low light condition was selected during the treatment in order to prevent excessive water loss by transpiration. The treatment was applied to three replicates for both wild-type and transgenic plants, and was continued for 6 days.

2.4 Measurements of gas exchange

The net photosynthetic rate (P_n) and stomatal conductance (g_s) of the seventh leaf from the apex were estimated in the artificial-light room with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with a blue-red light emitting diode as a light source (6400-02B, Li-Cor). The two activities were measured under PPFDs of 200 μmol photons m^{-2} s^{-1} and 60 μmol photons m^{-2} s^{-1} for poplar plants exposed to drought stress and salt stress, respectively. The concentration of CO_2 was 370 μmol mol^{-1}.

2.5 Measurements of ethylene production

The rate of ethylene production was measured as described by Bae et al. (1996). Leaves were excised from all plants used for the measurements of P_n and g_s after 4 days of drought stress and 5 days of salt stress, and incubated in sealed flasks under light for 1 h. Then, 1 mL of gas was withdrawn from each flask, and ethylene was analyzed using a gas chromatograph equipped with a flame ionization detector (GC-7 A; Shimadzu, Tokyo, Japan).

2.6 Measurements of chlorophyll content

Chlorophyll (Chl) content was determined spectrophotometrically according to Arnon (1949). After 5 days of drought stress, leaf discs were excised from the eighth leaf from the apex. Each frozen leaf disc was powdered by grinding in a mortar and pestle with liquid N_2, followed by further grinding with 80% acetone.

2.7 Quantification of *ACS* gene expression

The expression of the endogenous *ACS* gene in mature leaves was quantified by reverse transcription quantitative real-time PCR (RT-qPCR) using gene-specific primers. The upstream and downstream primer sequences were 5'-GAG AGT TAG AGG AGG AAG GGT GA-3' and 5'-GGA GAA GGA ACA AGG AAA GCA-3', respectively. For plants subjected to drought stress, a leaf disc was prepared from the same leaf that was used for measurement of Chl. For each plant subjected to salt stress, a leaf disc was prepared from the eighth leaf from the apex after 5 days of salt stress treatment. Total RNA was isolated from the discs by the hexadecyltrimethylammonium bromide-chloroform extraction procedure (Shinohara and Murakami 1996), and purified with a Wizard® SV Total RNA Isolation kit (Promega, Madison, WI, USA). First-strand cDNA was synthesized for qPCR under same conditions described previously (Nishiguchi et al. 2012). The expression level of the polyubiquitin gene (*PnUB1*) was used as a control.

3. Results

3.1 Responses to drought stress

We monitored the water content of sand cultures during the drought-stress treatment to examine whether the stress was imposed on each plant to the same extent. The water content decreased in the same manner in the pots of both wild-type and transgenic poplars after the stress experiment began (Fig. S1). This indicated that there was no difference in the intensity of drought stress between the wild-type and transgenic plants.

Under these conditions, differences in gas exchange characteristics and visible symptoms were investigated among the wild-type and transgenic poplars. The P_n and g_s values were not affected by 2 days of treatment. Complete arrest of photosynthesis and stomatal closure were observed in both wild-type and transgenic poplars after 4 days of drought stress (Fig. 1), when all plants showed bent petioles simultaneously. During the first 2 days of treatment, the g_s value was higher in the transgenic poplar than in the wild-

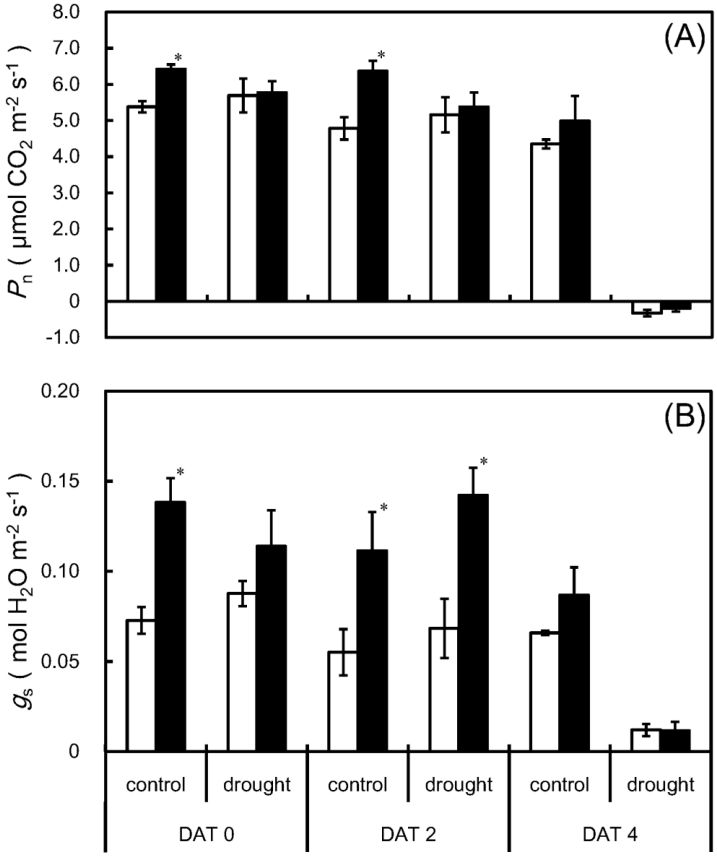

Fig. 1. Net photosynthetic rate, P_n (A) and stomatal conductance, g_s (B) responses of wild-type (white bars) and transgenic (black bars) poplars to drought stress. Gas exchange measurements on mature leaves under the control and drought treatments were carried out at 0, 2, and 4 days after treatment (DAT). Data represent means plus standard errors (n = 3). * indicates a significant difference between the wild-type and transgenic poplars in each treatment at $P < 0.05$ (t-test).

type irrespective of the stress treatment, while the P_n value was slightly higher in the transgenic poplar than in the wild-type under the control conditions. This was consistent with our previous results, in which transgenic tobacco with an antisense *ACS* DNA had a higher g_s value (Nakajima et al. 2002). Necrosis appeared and spread mainly in the mature leaves after 4 days of treatment. There was less leaf bronzing and defoliation in the transgenic poplar than in the wild-type (Fig. 2A). It is well known that foliar Chl breakdown can be accelerated by stress-induced senescence. In the present study, the total Chl content of mature leaves was decreased by the stress treatment, and was more pronounced in the wild-type poplar (Fig. 2B). Thus, drought-induced senescence was delayed in the transgenic poplar with an antisense *ACS* DNA.

To investigate the difference in the severity of leaf injury, the level of ethylene production was compared under drought stress. Although no ethylene was detected in either wild-type or transgenic poplar in the absence of stress, ethylene biosynthesis was induced under drought stress (Fig. 2C). The drought-induced ethylene production in the leaves of transgenic poplars was about 4 times lower than in those of the wild-type. The delay of drought-induced senescence in the transgenic poplar is attributable to the suppression of ethylene production. Similarly, the level of *ACS* gene expression under drought stress was lower in the transgenic poplar than in the wild-type (Fig. 2D). The similar patterns of ethylene production and gene expression suggest that the *ACS* gene plays an important role in ethylene production under drought stress.

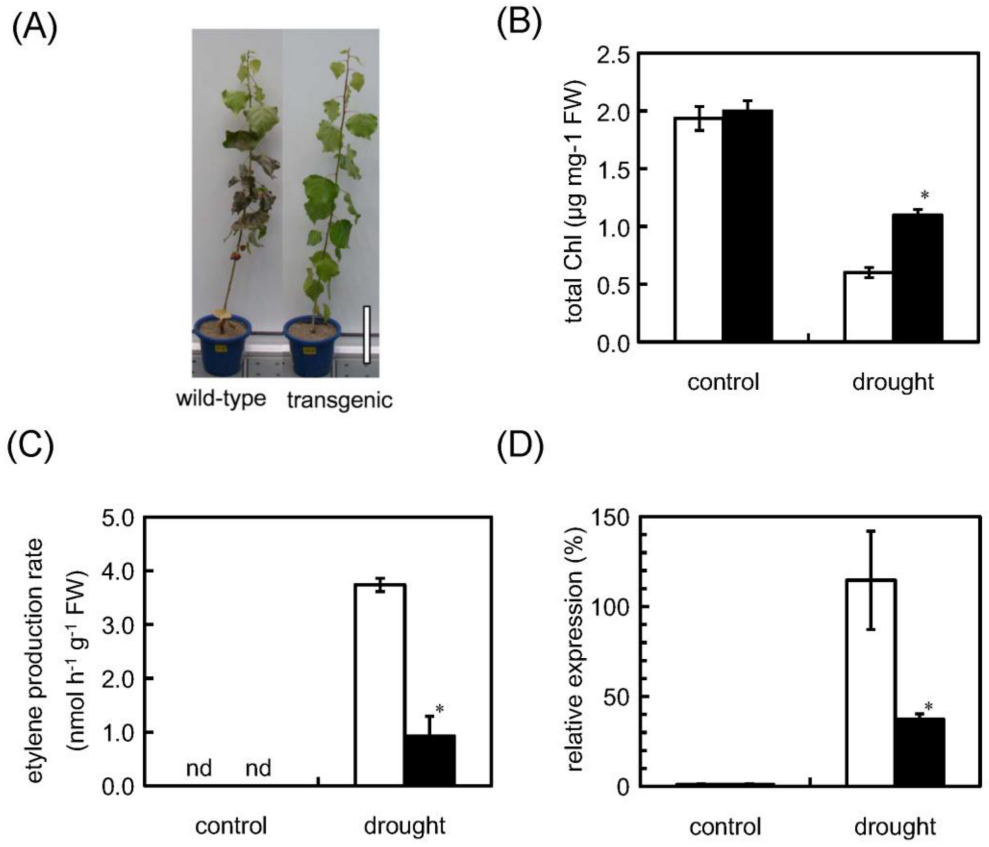

Fig. 2. Drought-induced damage of mature leaves and ethylene biosynthesis. (A) Visible foliar injuries in response to drought stress. On Day 5 of the treatment, leaf bronzing and defoliation was observed. Scale bar = 10 cm. (B) Decrease in total chlorophyll (Chl) content of mature leaves in wild-type (white bars) and transgenic (black bars) poplars under drought stress. Chl was extracted from mature leaves under the control and drought treatment. (C) Drought-induced ethylene production from mature leaves of wild-type and transgenic poplars. Mature leaves under the control and drought treatments were excised from each plant, and the ethylene they emitted was collected. (D) Drought-induced expression of endogenous *ACS* in mature leaves of wild-type and transgenic poplars. Total RNA was extracted from mature leaves under the control and drought treatments. The expression levels of *ACS* are expressed relative to the wild-type poplar under control conditions. All data represent means plus standard errors (n = 3). * indicates a significant difference between the genotypes in each treatment at $P < 0.05$ (t-test). nd indicates not detected.

3.2 Responses to salt stress

The salt stress treatment decreased P_n in both the wild-type and transgenic poplars (Fig. 3), but the inhibition of P_n was moderate in the transgenic poplar compared with the wild-type (Fig. 3A). P_n was completely inhibited in the wild-type plants after 2 days of salt stress (Fig. 3A). The g_s value was decreased by the treatment more slowly in the transgenic poplar than in the wild-type (Fig. 3B). After 6 days of salt stress, gas exchange rate was not measurable in the wild-type (Fig. 3B) because of extremely low g_s.

Visible injuries appeared on mature leaves after 6 days of salt stress but spread more slowly in transgenic poplars than in the wild-type (Fig. 4A). This result indicated that the impact of salt stress on leaf integrity was attenuated in the transgenic poplar with an antisense *ACS* DNA. As in the drought stress experiment, ethylene production was not detected under control conditions, but was induced in mature leaves by salt stress (Fig. 4B). The ethylene production under salt stress was lower in the mature leaves of transgenic poplar than in those of the wild-type (Fig. 4B). Therefore, we presume that the decrease in ethylene production could have beneficial effects on the tolerance of transgenic poplar to salt stress. The inducibility of ethylene production was consistent with the response of *ACS* expression to salt stress. Although salt-induced expression of *ACS* was observed in both the wild-type and transgenic poplars, it was induced to a lesser extent in the transgenic plants (Fig. 4C), suggesting that the *ACS* could be associated with both salt-induced and drought-induced ethylene production.

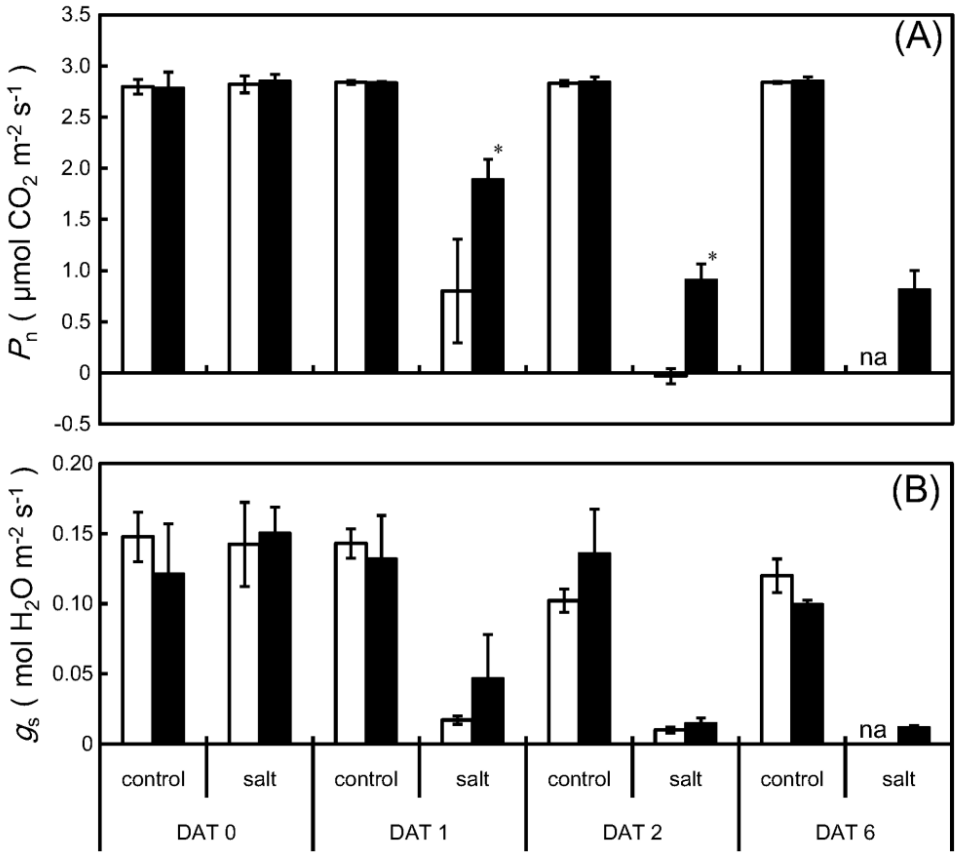

Fig. 3. Net photosynthetic rate, P_n (A) and stomatal conductance, g_s (B) responses of wild-type (white bars) and transgenic (black bars) poplars to salt stress. Gas exchange measurements on mature leaves under the control and salt treatments were carried out at 0, 1, 2 and 6 days after treatment (DAT). The data represent means plus standard errors (n = 3). * indicates a significant difference between the wild-type and transgenic poplars in each treatment at $P <$ 0.05 (t-test). na indicates data not available because of extremely low stomatal conductance.

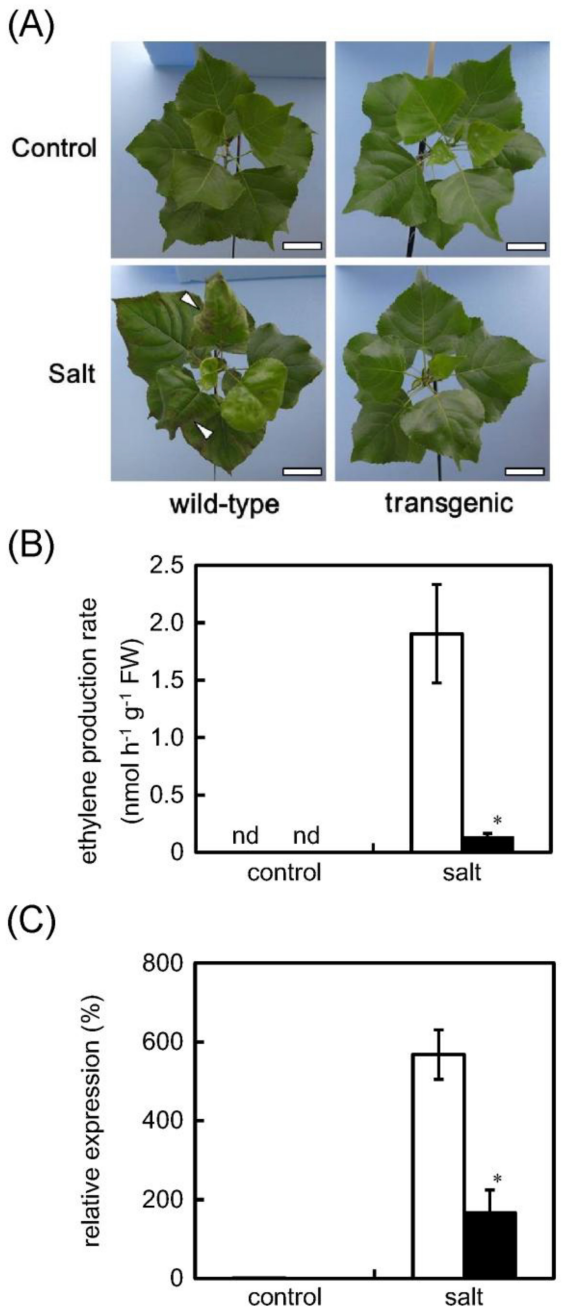

Fig. 4. Salt-induced damage of mature leaves and ethylene biosynthesis. (A) The phenotype of poplars seen from directly above. Under salt stress for 6 days, leaf edge necrosis was advanced in the wild-type (white arrowheads), but barely apparent in the transgenic poplar. (B) Salt-induced ethylene production from mature leaves of wild-type (white bar) and transgenic (black bar) poplars. Ethylene emitted from mature leaves under the control and salt treatments was collected. (C) Salt-induced expression of endogenous *ACS* in mature leaves of wild-type and transgenic poplars. Total RNA was extracted from mature leaves under the control and salt treatments. The expression levels of *ACS* are expressed relative to the wild-type poplar under control conditions. All data represent means plus standard errors (n = 3). * indicates a significant difference between the genotypes in each treatment at $P < 0.05$ (t-test). nd indicates not detected.

4. Discussion

Transgenic poplar with an antisense *ACS* DNA exhibited tolerance not only to ozone stress (Mohri et al. 2011), but also to drought and salt stresses as shown in the present study. Under drought stress, drought-induced senescence, which occurs after stomatal closure and photosynthetic inhibition (Fig. 1A, B), was moderate in the transgenic poplar (Fig. 2A, B). This trait should be advantageous, especially in conditions of transient water deficit since there are more chances to resume photosynthesis after re-watering. In response to salt stress, the transgenic poplar maintained photosynthetic activity (Fig. 3) and displayed only minor symptoms of necrosis (Fig. 4A). These results suggest that the transgenic poplar with an antisense *ACS* DNA has a significant growth advantage under salt stress.

In response to drought stress, the wild-type poplars showed a significant increase in ethylene production (Fig. 2C), as seen in other plant species, including wheat (Apelbaum and Yang 1981, Beltrano et al. 1999), alfalfa (Irigoyen et al. 1992) and jack pine (Rajasekaran and Blake 1999). Foliar ethylene production was also induced in response to salt stress (Fig. 4B), as observed in tomato (Feng and Barker 1992) and red pepper (Siddikee et al. 2011). An increase in ethylene biosynthesis has been implicated in drought-induced senescence, since loss of *ACS* expression delayed the onset of senescence during drought stress as well as natural senescence (Young et al. 2004). Inhibitors of ethylene biosynthesis retarded drought-induced senescence in wheat (Beltrano et al. 1999). In the present study, the transgenic poplar showed a phenotype characterized by the delay of drought-induced senescence, as indicated by less visible symptoms of leaf bronzing (Fig. 2A) and sustained Chl content (Fig. 2B). It is assumed that ethylene is responsible for drought-induced senescence in poplars as well as in other plant species. The phenotype of transgenic poplars can be attributed to reduced induction of ethylene biosynthesis under drought stress (Fig. 2C). Similarly, the foliar necrosis observed under salt stress appears to be associated with salt-induced ethylene biosynthesis, the level of which can be limited experimentally by controlling ACC content (Feng and Barker 1992, Siddikee et al. 2011). These findings are in agreement with our results, in which the mitigation of foliar necrosis in the transgenic poplar under salt stress (Fig. 4A) can be explained by reduced induction of ethylene biosynthesis (Fig. 4B).

Ethylene is an essential factor in the regulation of growth and development in response to environmental stresses (Wang et al. 2002) and in disease resistance (van Loon et al. 2006). Therefore, a severe block in ethylene

biosynthesis may have detrimental effects on plant integrity. It was reported that over-expression of a mutated ethylene receptor gene caused the premature death of plants (Shibuya et al. 2004). At least in the control conditions, our transgenic poplar showed no inhibition of growth (data not shown) or net photosynthetic rate (Fig. 1, 3). We speculate that a moderate reduction of stress-induced ethylene biosynthesis (Fig. 2C, 4B) is suitable for practical applications.

Ozone-induced ethylene biosynthesis in tomato is known to be linked to biphasic regulation of *ACS* genes (Moeder et al. 2002), with early induction of *LeACS6* and later induction of *LeACS2*. The putative orthologue of *LeACS2* in tobacco, *NtACS1*, also participates in ozone-induced ethylene biosynthesis (Samuel et al. 2005). Phylogenetic analysis revealed that *PoACS2* isolated from *P. deltoides* × *P. nigra*, an ozone inducible *ACS* gene (accession No. AB033503; Mohri et al. 2011), belongs to the same clade as *LeACS2* and *NtACS1* (data not shown). It has been reported that *LeACS2* is induced not only by ozone stress, but also by other environmental stimuli. For example, *LeACS2* expression was induced by wounding (Tatsuki and Mori 1999), a fungal elicitor (Matarasso et al. 2005) and flooding (Shiu et al. 1998). Similarly, the expression of *NtACS1* is enhanced in response to various stresses including salt (Cao et al. 2006; Wi et al. 2010), pathogen infection (Wi et al. 2012) and wounding (Wi et al. 2012). In the present study, the expression of poplar *ACS* gene was induced under drought (Fig. 2D) and salt stress (Fig. 4C), as well as under ozone stress (Mohri et al. 2011), indicating that the *ACS* gene is also a multiple stress-responsive gene. Moreover, our data suggest that the *ACS* could regulate the ethylene biosynthesis leading to foliar injury under multiple stresses.

In attempts to confer stress tolerance to plants, ethylene biosynthesis has been targeted for manipulation in many studies. In particular, genetic or pharmacological interference of ACC supply to ethylene biosynthesis seems to be effective in attenuating stress-induced injury or premature senescence. Treatment with aminoethoxyvinyl glycine, an inhibitor of ACS, can alleviate foliar damage under ozone stress (Tamaoki et al. 2003), drought stress (Beltrano et al. 1999) and ultraviolet-B irradiation (Nara and Takeuchi 2002). In addition, bacterial ACC deaminase, which hydrolyses ACC to ammonia and α-ketobutyrate, has been reported to ameliorate damage under stress conditions. In several plants, inoculation of rhizobacteria containing ACC deaminase may confer tolerance to salt stress (Mayak et al. 2004a; Siddikee et al. 2011) and drought stress (Mayak et al. 2004b) by decreasing the ACC level to suppress stress-induced ethylene biosynthesis. Moreover, transgenic canola expressing bacterial ACC deaminase exhibited higher tolerance to salt stress (Sergeeva et al. 2005). In the context of interference of ACC supply, together with previous findings (Mohri et al. 2011), our results showed that antisense expression of *ACS* is a good strategy to inhibit stress-induced ethylene biosynthesis (Fig. 2C, 4B), and results in mitigation of foliar injury under multiple stresses (Fig. 2A, 4A). This is comparable with a study using transgenic tobacco plants (Wi et al. 2010). Transgenic tobacco with antisense expression of carnation *ACS* showed reduced ethylene biosynthesis and accumulation of reactive oxygen species (ROS) in response to H_2O_2 treatment, and was less sensitive to H_2O_2 as well as salt stress (Wi et al. 2010), suggesting that inhibition of ACS could attenuate the synergistic effects between biosynthesis of ethylene and ROS. It is speculated that genetic modulation by down-regulation of *ACS* should confer improved tolerance to various abiotic stresses accompanied by increased ROS production. Therefore, our transgenic poplar is expected to exhibit higher tolerance to various other environmental stresses besides ozone, drought and salt stresses.

In conclusion, the present study shows that the introduction of an antisense *ACS* DNA effectively confers multiple stress tolerance to poplar, indicating that the *ACS* gene is responsive to multiple stresses and should participate in the maintenance of high-level ethylene production that causes leaf damage under those stresses. Further studies including the effects of modified ethylene biosynthesis on the viability of poplar will be required to assess the possible range of its application.

Acknowledgement

This research was supported by a research grant (No. 200906) from the Forestry and Forest Products Research Institute.

References

Apelbaum, A. and Yang, S. F. (1981) Biosynthesis of stress ethylene induced by water deficit. Plant Physiol., 68, 594–596.

Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol., 24, 1–15.

Bae, G. Y., Nakajima, N., Ishizuka, K. and Kondo, N. (1996) The role in ozone phytotoxicity of the evolution of ethylene upon induction of 1-aminocyclopropane-1-carboxylic acid synthase by ozone fumigation in tomato plants. Plant Cell Physiol., 37, 129–134.

Beltrano, J., Ronco, M. G. and Montaldi, E. R. (1999) Drought stress syndrome in wheat is provoked by ethylene evolution imbalance and reversed by rewatering, aminoethoxyvinylglycine, or sodium benzoate. J. Plant Growth Regul., 18, 59–64.

Cao, W., Liu, J., Zhou, Q., Cao, Y., Zheng, S., Du, B., Zhang, J. and Chen, S. (2006) Expression of tobacco ethylene receptor NTHK1 alters plant responses to salt stress. Plant Cell Environ., 29, 1210–1219.

Czarny, J. C., Grichko, V. P. and Glick, B. R. (2006) Genetic modulation of ethylene biosynthesis and signaling in plants. Biotechnol. Adv., 24, 410–419.

Feng, J. and Barker, A. (1992) Ethylene evolution and ammonium accumulation by tomato plants under water and salinity stresses. Part II. J. Plant Nutr., 15, 2471–2490.

Irigoyen, J. J., Emerich, D. W., and Sanchez-Diaz, M. (1992) Alfalfa leaf senescence induced by drought stress: photosynthesis, hydrogen peroxide metabolism, lipid peroxidation and ethylene evolution. Physiol. Plant., 84, 67–72.

Matarasso, N., Schuster, S. and Avni, A. (2005) A novel plant cysteine protease has a dual function as a regulator of 1-aminocyclopropane-1-carboxylic acid synthase gene expression. Plant Cell, 17, 1205–1216.

Mayak, S., Tirosh, T. and Glick, B. R. (2004a) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol. Biochem., 42, 565–572.

Mayak, S., Tirosh, T. and Glick, B. R. (2004b) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci., 166, 525–530.

Moeder, W., Barry, C. S., Tauriainen, A. A., Betz, C., Tuomainen, J., Utriainen, M., Grierson, D., Sandermann, H., Langebartels, C. and Kangasjärvi, J. (2002) Ethylene synthesis regulated by biphasic induction of 1-aminocyclopropane-1-carboxylic acid synthase and 1-aminocyclopropane-1-carboxylic acid oxidase genes is required for hydrogen peroxide accumulation and cell death in ozone-exposed tomato. Plant Physiol., 130, 1918–1926.

Mohri, T., Kogawara, S., Igasaki, T., Yasutani, I., Aono, M., Nakajima, N. and Shinohara, K. (2011) Improvement in the ozone tolerance of poplar plants with an antisense DNA for 1-aminocyclopropane-1-carboxylate synthase. Plant Biotechnol., 28, 417–421.

Nakajima, N., Itoh, T., Takikawa, S., Asai, N., Tamaoki, M., Aono, M., Kubo, A., Azumi, Y., Kamada, H. and Saji, H. (2002) Improvement in ozone tolerance of tobacco plants with an antisense DNA for 1-aminocyclopropane-1-carboxylate synthase. Plant Cell Environ., 25, 727–735.

Nara, A. and Takeuchi, Y. (2002) Ethylene evolution from tobacco leaves irradiated with UV-B. J. Plant Res., 115, 247–253.

Nishiguchi, M., Nanjo, T. and Yoshida, K. (2012) The effects of gamma irradiation on growth and expression of genes encoding DNA repair-related proteins in Lombardy poplar (*Populus nigra* var. *italica*). J. Environ. Radioactiv., 109, 19–28.

Pierik, R., Tholen, D., Poorter, H., Visser, E. J. W. and Voesenek, L. A. C. J. (2006) The Janus face of ethylene: growth inhibition and stimulation. Trends Plant Sci., 11, 176–183.

Rajasekaran, L. R. and Blake, T. J. (1999) New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. J. Plant Growth Regul., 18, 175–181.

Samuel, M. A., Walia, A., Mansfield, S. D. and Ellis, B. E. (2005) Overexpression of SIPK in tobacco enhances ozone-induced ethylene formation and blocks ozone-induced SA accumulation. J. Exp. Bot., 56, 2195–2201.

Sergeeva, E., Shah, S. and Glick, B. R. (2005) Growth of transgenic canola (*Brassica napus* cv. Westar) expressing a bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase gene on high concentrations of salt. World J. Microbiol. Biotechnol., 22, 277–282.

Shibuya, K., Barry, K. G., Ciardi, J. A., Loucas, H. M., Underwood, B. A., Nourizadeh, S., Ecker, J. R., Klee, H. J. and Clark, D. G. (2004) The central role of PhEIN2 in ethylene responses throughout plant development in petunia. Plant Physiol., 136, 2900–2912.

Shinohara, K. and Murakami, A. (1996) Changes in levels of thylakoid components in chloroplasts of pine needles of different ages. Plant Cell Physiol., 37, 1102-1107.

Shinohara, K., Nagao, A., Okuda, S., Niiyama, K. and Sugawa, T. (1998) Effects of temperature on the growth of a Japanese willow (*Salix gilgiana* Seemen). J. For. Res., 3, 55-60.

Shiu, O. Y., Oetiker, J. H., Yip, W. K. and Yang, S. F. (1998) The promoter of *LE-ACS7*, an early flooding-induced 1-aminocyclopropane-1-carboxylate synthase gene of the tomato, is tagged by a Sol3 transposon. Proc. Natl. Acad. Sci. USA, 95, 10334–10339.

Siddikee, M. A., Chauhan, P. S. and Sa, T. (2011)

Regulation of ethylene biosynthesis under salt stress in red pepper (*Capsicum annuum* L.) by 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase-producing halotolerant bacteria. J. Plant Growth Regul., 31, 265–272.

Tamaoki, M., Matsuyama, T., Kanna, M., Nakajima, N., Kubo, A., Aono, M.and Saji, H. (2003) Differential ozone sensitivity among Arabidopsis accessions and its relevance to ethylene synthesis. Planta, 216, 552–560.

Tatsuki, M. and Mori, H. (1999) Rapid and transient expression of 1-aminocyclopropane-1-carboxylate synthase isogenes by touch and wound stimuli in tomato. Plant Cell Physiol., 40, 709–715.

van Loon, L. C., Geraats, B. P. J. and Linthorst, H. J. M. (2006) Ethylene as a modulator of disease resistance in plants. Trends Plant Sci., 11, 184–191.

Wang, K. L. C., Li, H. and Ecker, J. R. (2002) Ethylene biosynthesis and signaling networks. Plant Cell, 14, 131–152.

Wi, S. J., Jang, S. J. and Park, K. Y. (2010) Inhibition of biphasic ethylene production enhances tolerance to abiotic stress by reducing the accumulation of reactive oxygen species in *Nicotiana tabacum*. Mol. Cells, 30, 37–49.

Wi, S. J., Ji, N. R. and Park, K.Y. (2012) Synergistic biosynthesis of biphasic ethylene and reactive oxygen species in response to hemibiotrophic *Phytophthora parasitica* in tobacco plants. Plant Physiol., 159, 251–265.

Yang, S. and Hoffman, N. (1984) Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 35, 155–189.

Young, T. E., Meeley, R. B. and Gallie, D. R. (2004) ACC synthase expression regulates leaf performance and drought tolerance in maize. Plant J., 40, 813–825.

Supplemental materials

Additional supplemental materials are provided in the online version.

Fig. S1. Sand water content in the control and drought treatments.

URL : http://www.ffpri.affrc.go.jp/pubs/bulletin/432/documents/432.pdf

オゾン耐性遺伝子組換えポプラの耐乾燥性および耐塩性

古川原 聡[1)]、毛利 武[2)*]、伊ヶ崎 知弘[2)]、中嶋 信美[3)]、篠原 健司[2)]

要 旨

エチレン合成のキイ酵素である 1-aminocyclopropane-1-carboxylate synthase (ACS) の遺伝子発現を抑制した遺伝子組換えポプラ (*Populus nigra* var. *italica* Koehne) の耐乾燥性と耐塩性を調べた。我々は、この組換えポプラが高いオゾン耐性を持つことを報告している。組換えポプラに乾燥ストレスを与えると、葉の老化や落葉が緩和された。また、塩ストレス下では、組換えポプラの葉の傷害は軽微であり、野生型ポプラと比べ光合成活性が高く維持されていた。乾燥ストレスと塩ストレスは、オゾンストレスと同様に、野生型ポプラの葉における ACS 遺伝子の発現を誘導し、エチレン合成を促進した。一方、組換えポプラでは ACS 遺伝子の誘導は阻害され、エチレン合成の促進も抑制された。したがって、ACS 遺伝子はこれらのストレスによって誘導されるエチレン合成を制御し、葉の老化や傷害を引き起こす役割を担っていると考えられる。このように、ACS 遺伝子の発現を抑制することで、複数のストレスに対するポプラの耐性を高めることができる。

キーワード：ACC 合成酵素、環境ストレス、セイヨウハコヤナギ、エチレン合成の抑制

原稿受付：平成 26 年 2 月 21 日　原稿受理：平成 26 年 6 月 19 日
1) 東京大学アジア生物資源環境研究センター
2) 森林総合研究所生物工学研究領域
3) 国立環境研究所生物・生態系環境研究センター
＊森林総合研究所生物工学研究領域　〒 305-8687　茨城県つくば市松の里 1

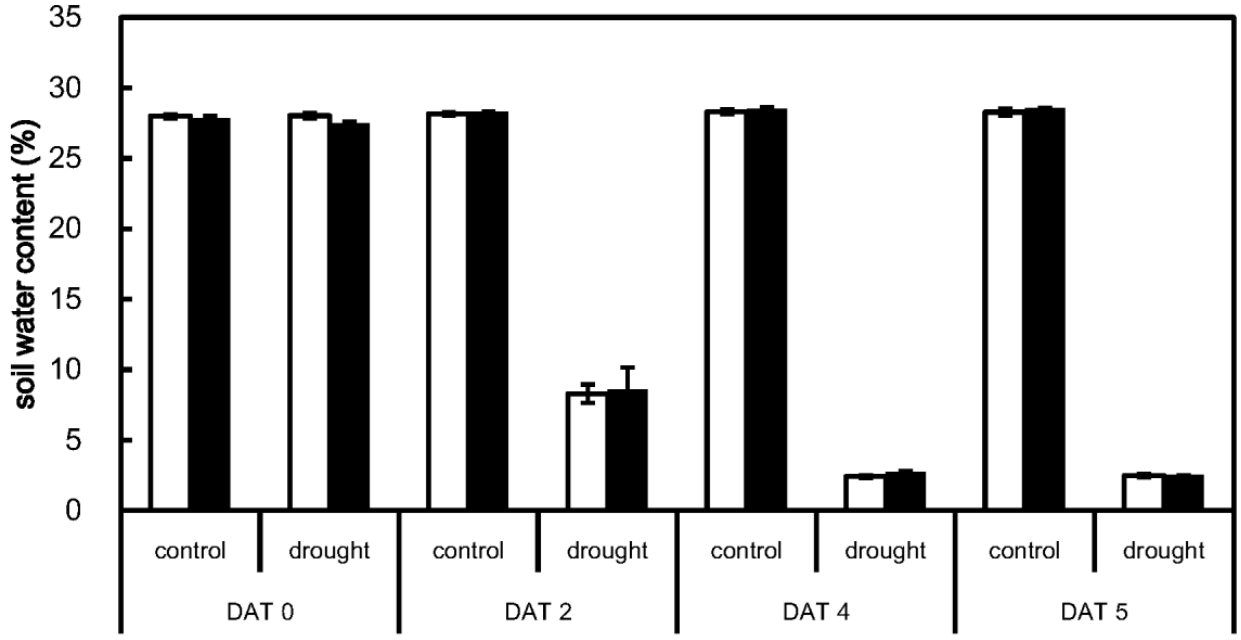

Fig. S1 Sand water content in the control and drought treatments. At 0, 2, 4 and 5 days after treatment (DAT), the water content was measured by inserting a probe into all pots of wild-type (white bars) and transgenic (black bars) poplars. Data represent means plus standard errors (n = 3).