Studies for Absorption of Formaldehyde by Using Foliage on Wild Tomato Species

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Formaldehyde (HCHO) absorption capacity (indicating that HCHO was absorbed into foliage) was measured. Then, the metabolism-related substances (glutathione: GSH) and enzymes (glutathione-dependent formaldehyde dehydrogenase: FALDH and formate dehydrogenase: FDH) in the foliage of wild tomato species were investigated histochemically. In the measurement of HCHO absorption capacity, fresh foliage explant, which was placed in a sealed glass container, was treated with the adjusted 5 ppm HCHO from outside. As a result, in Lycopersicon (Solanum) pennellii LA0716, the HCHO concentration in the glass container significantly decreased down to 0.08 ppm, which is a guideline value indicating the safety of indoor air concentration established by the World Health Organization (WHO). On the other hand, the HCHO concentration of L. chilense TOMATO(WILD)94 did not decrease to the guideline value. Therefore, these results showed that LA0716 was an HCHO “high-absorbing” type in terms of capacity to remove HCHO, and TOMATO(WILD)94 was a “low-absorbing” type. An interspecific difference was observed among the wild tomatoes that were used in this study. In addition, changes in localization of HCHO metabolism-related substances and enzyme activity during treatment with HCHO was observed in the HCHO “high-absorbing” type, but was not shown in the HCHO “low-absorbing” type for each passage of time after HCHO treatment. In our study, we showed the possibility that fresh foliage explant in one wild tomato species, L. (S.) pennellii, absorbed and metabolized “toxic” HCHO.

Key Words: formate dehydrogenase, glutathione, glutathione-dependent formaldehyde dehydrogenase, phytoremediation, wild tomato species.

Introduction

The living environment of modern houses has progressed and become very airtight with good thermal insulation. Closed spaces have increased in order to increase the energy efficiency such as cooling and heating as energy-saving measures. In Japan, the residential style changed from the traditional wooden Japanese house to the reinforced concrete house in the western-style, and building materials also began to change from natural materials such as wood to synthetic materials containing chemical substances such as plywood and other inner materials (Osawa, 2010). However, volatile organic compounds (VOCs) such as formaldehyde (HCHO), toluene, and xylene are generated from these synthetic materials. VOCs were also generated from coating materials for administering indoor paint in the reinforced concrete houses in the western-style. It is well known that the VOCs have become the cause of sick house syndrome, which has symptoms of headache, dizziness, dry cough, difficulty breathing, and mental anxiety. It became a social problem due to the resulting harm to human health (Seki et al., 2007). Since the deterioration of indoor environments due to these VOCs became a problem, the World Health Organization (WHO) set indoor concentration guideline values. One of the VOCs, the HCHO concentration value, was set at 0.08 ppm indoors (Kaden et al., 2010).

Plants can remove VOCs in air that adversely affects the human body, and the VOCs removed from air are translocated to the roots, then decomposed by microorganisms in the rhizosphere on soil (Wolverton, 1996; Wolverton and Wolverton, 1993). The air purification...
technology that utilizes plants is called phytoremediation (Salt et al., 1998). However, it has not been confirmed that the microorganisms in the soil actually decompose the VOCs which plants translocate to the roots.

As for research into the HCHO removal effect by using plants, pothos (Epipremnum aureum, Onodera et al., 1999), and golden pothos (Oyabu et al., 2001, 2003; Sawada et al., 2002) have been reported. In these reports, the HCHO removal capacity of pothos was affected by the type of plant and pollutants, or cultivation environmental factors such as temperature, illuminance, and soil type. Although the HCHO removal capability of pothos was reported in this research, it was not mentioned whether HCHO was decomposed and metabolized in the plant tissue. In addition, when the microorganisms in the soil decomposed HCHO, one concern is that the HCHO was absorbed into water on soil given for the irrigation or re-released when the soil dries.

Uotila and Koivusalo (1974a, b) reported the HCHO metabolic pathway in the human liver (Fig. 1). In this pathway, absorbed “toxic” HCHO in the human liver formed S-hydroxymethylglutathione combined with glutathione (GSH) and localized intracellularly at first. Then, it dehydrogenated to S-formylglutathione by glutathione-dependent formaldehyde dehydrogenase (FALDH). After that, S-formylglutathione was hydrolyzed to formic acid and GSH by S-formylglutathione hydrolase, and finally metabolized to “non-toxic” CO$_2$ by formate dehydrogenase (FDH). Moreover, localization of GSH and FALDH showed high activity related to the HCHO metabolism site in the rat liver histochemically (Keller et al., 1990). As for research into HCHO metabolism in plants, $^{14}$C-labelled formaldehyde which was exposed to leaves of golden pothos and weeping fig (Schmitz et al., 2000) and pea seeds (Uotila and Koivusalo, 1979) was reported. In these reports, $^{14}$C-labelled formaldehyde became $^{14}$CO$_2$ after a two step enzymatic oxidation process by formaldehyde and formate dehydrogenase. Characterization of formaldehyde dehydrogenase involved in the metabolism of HCHO was reported in Arabidopsis (Martinez et al., 1996), dried peas (Shafaqat et al., 1996), and spider plants (Giese et al., 1994). The genes for FALDH and HCHO-responsive genes were isolated in Arabidopsis and golden pothos, respectively (Dolferus et al., 1997; Tada and Kidu, 2010). However, histochemical studies that capture the reaction of substances related to HCHO metabolism are unknown in plant tissues.

Wild tomato species include nine known species distributed in Peruvian Andes in South America and the Galapagos islands (Taylor, 1986). These species have many characteristics in terms of plant shape, and their functional reactions show rich diversity among natural habitats. Thus, wild tomato species have become very important germplasm resources for cultivated tomato breeders, and efforts have focused on preserving and multiplying useful species (Tabuchi and Arai, 1998; Tabuchi et al., 2000a, b, 2004, 2007). The tomato genome sequence has been completed (Tomato Genome Consortium, 2012), and it is now easy to analyze the various tomato functions. If it is possible to understand the phenomenon of HCHO metabolism, it will lead to the identification of substances, enzymes and genes involved in HCHO metabolism, and any the identified genes could be applied to other plant species. Moreover, diversified wild tomato species are very likely to respond more sensitively to HCHO than cultivated tomatoes. Therefore, wild tomato species are a suitable study resource. In this study, we investigated the HCHO absorption capacity and histochemically observed the localization of substances and enzyme activity related to the HCHO metabolic pathway, as proposed by Uotila and Koivusalo (1974a, b), by using foliage of wild tomato L. (S.) pennellii and L. chilense.

Materials and Methods

Plant materials

HCHO absorption capacity of plants of the wild tomato species L. cheesmansi, L. chilense, L. chmielewskii, L. esculentum var. cerasiforme, L. hirsutum, L. parviflorum, L. peruvianum, L. (S.) pennellii, and L. pimpinellifolium, a total of 81 accessions, was previously measured (data not shown). Especially, plants of wild tomato species L. (S.) pennellii and L. chilense showed significantly high and low HCHO absorption, respectively. The accession number of L. (S.) pennellii is LA0716, and
L. chilense is TOMATO(WILD)94 (Fig. 2). These accessions were used for this study. Each of these species is distributed in the Peruvian Andes in South America. Seeds of these wild tomato species were sown in peat moss (Sakata Seed Co., Nara, Japan) in February 2015, and transplanted in rock wool (100 mm × 100 mm × 100 mm, Nippon Rock Wool Co., Tokyo, Japan). The true leaf expanded to 4 leaves, and plants were grown in the greenhouse of Tamagawa University, Machida, Tokyo, Japan from 2015 to 2016. Seeds of the wild tomato species L. (S.) pennellii were supplied by C. M. Rick’s Tomato Genetics Resource Center, Department of Vegetable Crops, University of California, Davis and L. chilense seeds were supplied by NIAS Genebank, Japan.

Adjustment of plants to measure HCHO and CO₂ concentrations

Foliage at the moderate stage of leaf growth was collected from each plant and adjusted to a fresh weight of about 0.4 g. Then, the collected fresh foliage explant was inserted into a 4.0 mL screw vial (No. 1; Maruemu Co., Osaka, Japan) that contained distilled water, and the gap between the screw vial and the foliage explant was plugged using parafilm (Bemis Company, Inc., WI, USA) (Fig. 3).

Preparation of HCHO concentration for foliage treatment

A glass container (800 mL, SEISHO Co., Ltd., Tokyo, Japan) containing 10 mL formaldehyde solution (assay: 35.0–38.0%, Wako Pure Chemical Industries, Ltd., Osaka, Japan,) was shaken with a shaker (Eyela Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 196 rpm, 30 min, 25 ± 0.5°C temperature in order to volatilize the HCHO in the glass container. Then, 2 mL of the air in the glass container was aspirated with a syringe (VICI precision Sampling, Inc., LA, USA). The concentration at the time of HCHO treatment was adjusted to 5 ppm by this operation.

**HCHO and CO₂ concentration measurement**

HCHO was processed using a syringe for HCHO or a CO₂ measuring device (Fig. 4). We previously measured the capacity of HCHO absorption over a period of one week. As for LA0716, the HCHO concentration in the glass container was 0 ppm at 120 min after HCHO treatment and this HCHO concentration did not change until the end of the experiment. On the other hand, in TOMATO(WILD)94, the HCHO concentration in the glass container was constant and did not decrease after the HCHO treatment. In this study, HCHO concentration in the glass container was measured after 0, 30, 60, 90, and 120 min, and the CO₂ concentration after 0, 30, 60, 90, and 120 min using a Gas sampling pump GV-100 (GASTEC Co., Osaka, Japan) attached to a HCHO detector tube (No. 91L or No. 91LL; GASTEC Co.) or CO₂ detector tube (No. 2LC; GASTEC Co.). When the HCHO or CO₂ concentration was measured, the measuring device of HCHO or CO₂ was placed in the experimental room (25 ± 0.3°C, 9.69 ± 0.03 μmol·m⁻²·s⁻¹). HCHO and CO₂ concentration values in

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**Fig. 2.** Plants of wild tomato species used in this study (left side: leaf, right side: fruit). (A) and (B): L. (S.) pennellii LA0716, (C) and (D): L. chilense TOMATO(WILD)94.

**Fig. 3.** Foliage of wild tomato adjusted to measure HCHO or CO₂ concentration in this experiment.
this study are indicated by the average and standard error. Measurement of HCHO and CO₂ concentration in each wild tomato was repeated more than ten times using five leaves randomly selected from ten plants. In these experiments, five glass containers were used for each wild tomato and the experiment was done simultaneously.

**Histochemical detection of HCHO metabolism-related substances and enzymes**

The fresh foliage explant treated with the HCHO was immediately taken out of the glass container at processing times of 0, 30, 60, 90, and 120 min after HCHO treatment. Then, 30 μm fresh cross sections were cut by an automatic plant microtome (MT-3; Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan). For histochemical detection of GSH, fresh cross sections were immersed for 2 h in 2 mL GSH reaction reagent in the dark. The GSH reaction reagent was prepared based on Hartmann et al. (2003). For detection of GSH, a fluorescence microscope (Olympus BX51; Olympus Co., Tokyo, Japan) was used for observation with ultraviolet irradiation (excitation wavelength: 350–385 nm). For histochemical detection of FALDH and FDH activity, the fresh cross sections were immersed for 1 h in 2 mL reaction reagent to assess enzyme activity in a water bath maintained at 37°C constant-temperature. This reaction reagent was prepared based on Diaz et al. (2004). A light microscope (Olympus BX51) was used for observation of detected FALDH and FDH activity. In these histochemical detections, five leaves for each processing time after HCHO treatment were used.

**Results and Discussion**

**HCHO concentration in the glass container after HCHO treatment**

If the HCHO concentration in the glass container decreased when treated with HCHO, this implied that HCHO was absorbed into the fresh foliage explant. As for LA0716 (Fig. 5), the HCHO concentration in the glass container gradually decreased with time after HCHO treatment, and finally reached 0.16 ± 0.03 ppm at 120 min after HCHO treatment. Under the conditions in our experiment (fresh foliage explant weight: about 0.4 g, grass container volume: 800 mL, treated HCHO concentration: 5 ppm, experimental room environment: 25 ± 0.3°C, 9.69 ± 0.03 μmol·m⁻²·s⁻¹), the HCHO concentration in the glass container with fresh foliage explant was close to the 0.08 ppm, which is the guideline value for the safety of indoor air concentration established by the WHO. On the other hand, in

**Fig. 4.** Measuring device of HCHO or CO₂ concentration. It was confirmed by a pre-experiment that HCHO did not occur using this device. The device consisted of an 800 mL glass container (SEISHO Co., Ltd. Tokyo, Japan). A silicon cap and tube were attached to a detector tube passage site and HCHO injection site, respectively. This stopped leakage of air from the glass container. Each site had vinyl tape or a clip to reliably prevent leakage of air form the glass container. Fresh foliage explant placed in a sealed glass container was treated with the adjusted 5 ppm HCHO from outside.

**Fig. 5.** Changes in HCHO concentration in the glass container in different wild tomato species. Vertical bars indicate the standard error from each mean value. The glass container was only treated with HCHO without fresh foliage explant. Different letters indicate significant differences between the wild tomato species at P < 0.05 by Mann-Whitney U test.
TOMATO(WILD)94 (Fig. 5), the HCHO concentration in the glass container did not decrease with the passage of the time, and finally remained at 4.04 ± 0.58 ppm at 120 min after HCHO treatment. The remaining high HCHO concentration in the glass container of TOMATO(WILD)94 is considered toxic to humans.

In this experiment, the HCHO concentration in the glass container without fresh foliage explant did not change from 5 ppm (Fig. 5). This showed that treated HCHO did not adhere to foliage or the device. As a result, “toxic” HCHO could be absorbed by fresh foliage explant. As for the capacity of HCHO absorption, we think that LA0716 is a “high-absorbing type”, and TOMATO(WILD)94 is a “low-absorbing type”. Thus, for absorption of HCHO, an interspecific difference was observed between the wild tomato species that were used in this study. Results of high or low HCHO absorption type were also obtained for other wild tomato species and accessions (data not shown).

**CO₂ concentration in the glass container after HCHO treatment**

In LA0716 (Fig. 6), the CO₂ concentration in the glass container gradually increased up to 120 min after the HCHO treatment more than the CO₂ concentration in the fresh foliage explant untreated with HCHO. A significant difference in CO₂ concentration in the glass container was observed between LA0716 (+HCHO) and LA0716 (~HCHO) at 120 min. This result showed that the increase in CO₂ concentration up to 120 min was due to HCHO treatment of the fresh foliage explant. On the other hand, the CO₂ concentration in the glass container of TOMATO(WILD)94 was not observed as in LA0716, and a significant difference in CO₂ concentration in the glass container was observed between LA0716 and TOMATO(WILD)94 at 120 min after HCHO treatment (Fig. 6). According to Akimoto et al. (2001, 2002), promotion of respiration in seeds of...
woody plants is caused by acetaldehyde. Especially, this phenomenon was observed in the seeds of woody plants that store carbohydrate as a storage nutrient. Also in this study, it was inferred that HCHO absorbed in fresh foliage explant promoted respiration by using carbohydrate from mesophyll tissues as a substrate and emission of CO\(_2\) from fresh foliage explant also increased relatively. Therefore, CO\(_2\) concentration up to 120 min in LA0716 (+HCHO) included respiration of the fresh foliage explant. These results indicated that “toxic” HCHO was absorbed into the fresh foliage explant.

Localization of HCHO metabolism-related substances and enzyme activity

As for localization and detection of GSH, strong fluorescence was observed in the cytoplasm of the cells that constructed a leaf palisade and sponge tissues in LA0716. Especially, the leaf mesophyll tissue showed strong blue-green fluorescence at 0 and 30 min after HCHO treatment (Fig. 7: GSH). In the subsequent 60, 90, and 120 min after HCHO treatment, the extent of the blue-green fluorescence in the cytoplasm of the palisade and sponge tissues of leaves disappeared. In a study of cell-specific measurement of cytoplasm GSH in poplar leaves, cytoplasm GSH was detected histochemically, and it was confirmed that the localization of GSH appeared as blue-green fluorescence by using monochlorobimane (MCB) (Hartmann et al., 2003). Therefore, the blue-green fluorescence observed in the mesophyll tissue in LA0716 was recognized as GSH in this experiment. According to Uotila and Koivusalo (1974a, b), HCHO combined with GSH to form S-hydroxymethylglutathione in the HCHO metabolic pathway in the human liver. This indicated that detected GSH at 0 and 30 min after HCHO treatment was able to bind the HCHO absorbed by the foliage. Disappearance of the localization of GSH in the cells of the leaf palisade and sponge tissues at 60, 90, and 120 min after HCHO treatment implies that S-hydroxymethylglutathione may be formed in these tissues (Fig. 1: phase 1).

In LA0716, the leaf mesophyll tissue showed as a blue-violet precipitate as FALDH and FDH were activated. FALDH activity was observed in mesophyll tissues. Especially, a strong active site was observed in the cytoplasm of cells that constructed the leaf palisade and sponge tissue at 30 and 60 min (Fig. 7: FALDH). In the HCHO metabolic pathway in the human liver, S-hydroxymethylglutathione was dehydrogenated to become S-formylglutathione (Uotila and Koivusalo, 1974a, b). To study of formaldehyde dehydrogenase, nitro blue tetrazolium was used for histochemical detection of FALDH in rat tissues (Keller et al., 1990) and Arabidopsis thaliana seedlings (Diaz et al., 2004; Espunya et al., 2006). These reports suggest that the active site of FALDH shows a blue formazan precipitate. Thus, our results indicated that S-hydroxymethylglutathione may be dehydrogenated to become S-formylglutathione (Fig. 1: phase 2).

FDH activity began to be observed in leaf palisade and sponge tissue 60 min after treatment. In the subsequent 90 and 120 min, strong FDH activity was gradually observed in leaf mesophyll tissues in LA0716. The FDH active site was observed in cells of the leaf palisade and sponge tissue (Fig. 7: FDH). According to a study of FDH in plants (Alekseeva et al., 2011), FDH in plants is the NAD+-dependent type. Therefore, FDH in LA0716 may be recognized as an NAD+-dependent type. Observed strong FDH activity at 60–120 min after HCHO treatment indicated that formic acid may be dehydrogenated to CO\(_2\) (Fig. 1: phase 4). In contrast to LA0716, localization of GSH, and FALDH, and FDH activity were not observed at any times after HCHO treatment in TOMATO(WILD)94 (Fig. 8). A similar result was obtained for localization of GSH, FALDH, and FDH activity in other wild tomato species and accessions that showed high or low HCHO absorption types (data not shown). This result indicated that HCHO metabolism-related substances and enzyme activity in the foliage were different among absorbing types of HCHO.

Our experiments suggested that the LA0716 absorbed “toxic” HCHO in the air into the fresh foliage explant, metabolized it in this place and finally released a “non-toxic” CO\(_2\) from the foliage to the air, because localization of GSH, FALDH, and FDH activity related to HCHO metabolism in the mesophyll tissue were observed by histochemical detection with the decrease in the HCHO concentration in the glass container. HCHO metabolism in LA0716 would have followed the same pathway as HCHO metabolic pathways in the human liver proposed by Uotila and Koivusalo (1974a, b). Therefore, we conclude that the air polluted with HCHO was purified by fresh foliage explant in our experiment.

Previous reports have suggested that plants absorbed HCHO in the air and translocated it to the root zone, with the HCHO then decomposed by microorganisms in the rhizosphere on soil (Wolverton and Wolverton, 1993). Moreover, there is a high possibility that HCHO is absorbed in water on soil. However, according to our experiments, we newly propose that plants have the ability to absorb and metabolize “toxic” HCHO in the air by plant organ parts. This paper showed that HCHO was absorbed and metabolized in fresh foliage explant by detecting the substances and enzymes involved in HCHO metabolism. In addition to this study, it was indicated that HCHO was metabolized by plant organs such as seeds and leaves by detecting the enzymatic activity related to metabolism of HCHO (Schmitz et al., 2000; Uotila and Koivusalo, 1979). Transgenic plants overexpressing FALDH such as Arabidopsis and golden pothos showed an increased efficiency to take up exog-
enous HCHO as compared with wild type plants (Achkor et al., 2003; Tada and Kidu, 2011). Therefore, our research also supported the notion that the HCHO absorbed in plants was not decomposed by microorganisms in the rhizosphere on soil, but was metabolized by plant organs.

Histochemical techniques in this study may be useful to search for HCHO “high-absorbing” plants. Foliage of scented geraniums (Pelargonium spp.) used as ornamental or horticultural indoor plants, gave the same results (unpublished). Our approach is applicable to other ornamental indoor plants that have been reported to absorb “toxic” HCHO by Wolverton (1996).

The wild tomato L. (S.) pennellii LA0716 has been used in crosses for development of introgression lines (Eshed and Zamir, 1994). The introgression line was a mean to identify the gene involved in the absorption and metabolism of HCHO. Thus, the HCHO-metabolizing mechanism in foliage can be clarified by using an introgression line. L. (S.) pennellii, which is distributed in the Peruvian Andes, is an important germplasm resource, because L. (S.) pennellii has been reported to have useful functions such as resistance to the greenhouse whitefly (Gentile et al., 1968) and high levels of soluble solid contents (Tabuchi et al., 2004) for cultivated tomato breeders.

Our research has shown the wild tomato species L. (S.) pennellii can purify “toxic” HCHO in the air. Moreover, the plant itself has the ability to absorb and metabolize “toxic” HCHO as suggested by our research. We also contributed to the progress of HCHO metabolic studies in plants by using wild tomatoes.

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