SELECTIVE UPTAKE OF POLYCHLORINATED BIPHENYLS BY *CUCURBITA PEPO*

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Introduction

Members of the Cucurbitaceae family, which includes cucumbers, melons, pumpkins, squashes, and zucchini, are major global vegetable crops. However, there have been many reports of contamination of their fruits with hydrophobic organic pollutants such as *p,p*'-dichlorodiphenyldichloroethylene (DDE)[1], chlordane[2], dieldrin[3], and dioxins such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)[4]. Interestingly, the contamination is restricted to the Cucurbitaceae family[5], and no detailed molecular mechanisms for this selective contamination have been reported. Several critical steps are thought to be required for contamination of crops by hydrophobic compounds. First, the compounds must be desorbed from the soil matrix and solubilized, because they are usually bound to soil organic matter, which decreases their bioavailability. Low-molecular-weight organic acids such as citric and malic acids exuded from zucchini and cucumber roots facilitate the desorption of *p,p*'-DDE from weathered soil[6,7]. Next, the compounds must be taken up by plant roots. Hydrophobic compounds desorbed in the rhizosphere of plants are adsorbed on the roots and then move to the stele of the roots[8]. Translocation from the roots to the aerial parts via the xylem sap is also an important step, as indicated by the detection of the hydrophobic pollutants heptachlor epoxide and chlordane in the xylem sap of zucchini and cucumber[2]; genera-specific patterns of enantiomers were observed in the xylem sap and aerial parts of the plants, but not in the roots. Furthermore, Greenwood *et al.* reported congener-selective absorption and translocation of polychlorinated biphenyls (PCBs) through the xylem sap of *Cucurbita pepo*[9].

In this study, we compared the congener-selective accumulation of PCDDs, PCDFs, and PCBs in three *C. pepo* cultivars and in tobacco (Solanaceae) plants and evaluated the accumulation patterns of the compounds.

Materials and methods

Plant materials

Nicotiana tabacum L. cv. Samsun NN (tobacco) was used as a representative non-Cucurbitaceae plant. Seeds of *C. pepo* L. subsp. *ovifera* cv. Patty Green (PG) and subsp. *pepo* cv. Gold Rush (GR) were purchased from Johnny's Selected Seeds (Albion, ME, USA). Seeds of *C. pepo* subsp. *pepo* cv. Black Beauty (BB) were purchased from Tanenomori (Saitama, Japan).

Cultivation conditions

Approximately 1-month-old aseptically grown tobacco plants (n = 5) and 1-week-old *C. pepo* seedlings (PG, n = 4; BB, n = 5; GR, n = 5) were grown in soil contaminated with 7 PCDD congeners, 10 PCDF congeners, and 12 PCB congeners [5100 pg-toxic equivalent (TEQ) g^{-1}] for 32 days in a greenhouse[10]. Aerial parts were collected for quantification of the dioxins and dioxin-like compounds in terms of toxic equivalency factors (TEF).

Quantification of dioxins and dioxin-like compounds in the aerial parts of the plants

The dioxins and dioxin-like compounds were extracted from the plant samples and then concentrated, purified, and quantified by means of high-resolution gas spectrometry combined with high-resolution mass spectrometry, as reported previously[10].

Results and discussion

After 1 month of growth in contaminated soil, the aerial parts of the tobacco plants and the three *C. pepo* cultivars showed clear differences in the total concentrations of PCDDs, PCDFs, and PCBs (Figure 1). The compounds accumulated to much higher concentrations in the BB and GR aerial parts than in the tobacco and PG aerial parts. These results based on their concentrations are similar to those based on TEQ values[10]. Differences in the accumulation of dieldrin and endrin between non-Cucurbitaceae plants such as tobacco and Cucurbitaceae plants such as zucchini have been observed previously[5]. Taken together, all these results indicate that some members of the Cucurbitaceae family have a crucial feature that gives them the ability to efficiently take up hydrophobic compounds. Interestingly, the concentrations in the PG plants were almost the same as the concentrations in the tobacco plants, suggesting that PG has lost its uptake ability because almost all Cucurbitaceae plants can take up them. White *et al.* and our group reported differences in the accumulation of *p,p*'-DDE, dioxins, and dioxin-like compounds among *C. pepo* subspecies[6,11]. In addition to PG, other cultivars of the subspecies *ovifera* may have partially lost the ability to take up these compounds.

When we grew the plants hydroponically in the presence of dioxins and dioxin-like compounds, the compounds accumulated to significantly higher concentrations in the aerial parts of GR plants than in those of PG plants[12]. Because desorption of the compounds from soil organic matter is not necessary in hydroponic culture, their translocation from the roots to the aerial parts must have been partially responsible for the high accumulation.

Uptake efficiency is indicated by bioconcentration factors (BCFs), which are calculated by division of the concentrations of compounds in the aerial parts of plants by the concentrations in soil. By calculating BCFs, we determined that BB and GR plants took up PCDDs, PCDFs, and PCBs much more efficiently than did tobacco and PG plants (Figure 2). As the trends in accumulation of PCDDs, PCDFs, and PCBs were almost the same among plants tested, it is likely that BB and GR have factors that increased their uptake of these compounds. In all the plants, PCBs were taken up much more efficiently than were PCDDs and PCDFs, whereas PCDFs are primary contaminants in their concentrations in soil[10]. BB and GR plants took up approximately 4% of the PCBs

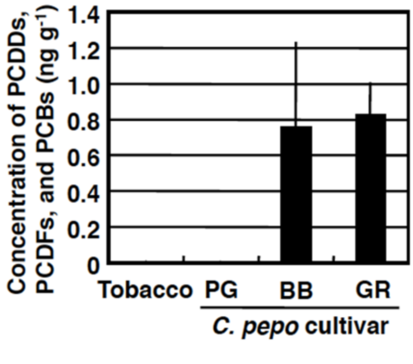

Figure 1 PCDD, PCDF, and PCB concentrations in tobacco plants and three *C. pepo* cultivars grown in soil contaminated with dioxins and dioxin-like compounds.
Concentrations of PCDDs, PCDFs, and PCBs with TEF values are totally calculated.

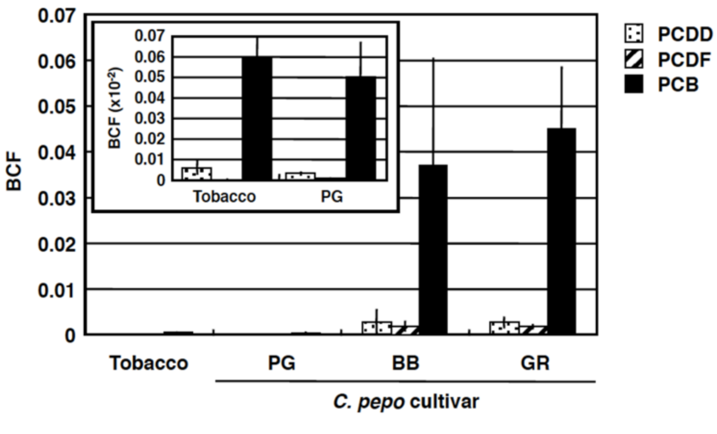

Figure 2 BCFs for PCDDs, PCDFs, and PCBs in tobacco plants and three *C. pepo* cultivars grown in soil contaminated with dioxins and dioxin-like compounds.
BCFs were calculated by dividing the concentration of each PCDD, PCDF, or PCB in the aerial parts of a plant by the corresponding concentration in soil. The inset shows a magnification of the tobacco and PG data.

in the soil. The mechanism for the efficient uptake of PCBs by plants is not clear.

The BCF patterns of the individual PCDD and PCDF congeners were similar in all plants (Figure 3A, B). Specifically, the BCFs for the tetrachlorinated congeners were higher than those for the penta-, hexa-, hepta-, and octachlorinated congeners. Negative correlations between the BCFs for the PCDD and PCDF congeners and the logarithms of the octanol–water partition coefficients (K_{ow}) were observed; highly chlorinated congeners are usually highly hydrophobic and therefore show low bioavailability[10,13,14]. In contrast, the BCF patterns for the PCB congeners in the BB and GR plants showed two large peaks, whereas only a small peak at 2,3',4,4',5-pentachlorobiphenyl was observed for the tobacco and PG plants (Figure 3C). All three of these peaks corresponded to *ortho*-chlorinated biphenyl congeners. The BCF peak corresponding to the highly chlorinated congeners was lower than the peak corresponding to the less chlorinated congeners, probably because uptake was decreased by the increase of hydrophobicity with increasing chlorination. These results suggest that the BB and GR plants accumulated *ortho*-chlorinated congeners by some unique mechanism that was

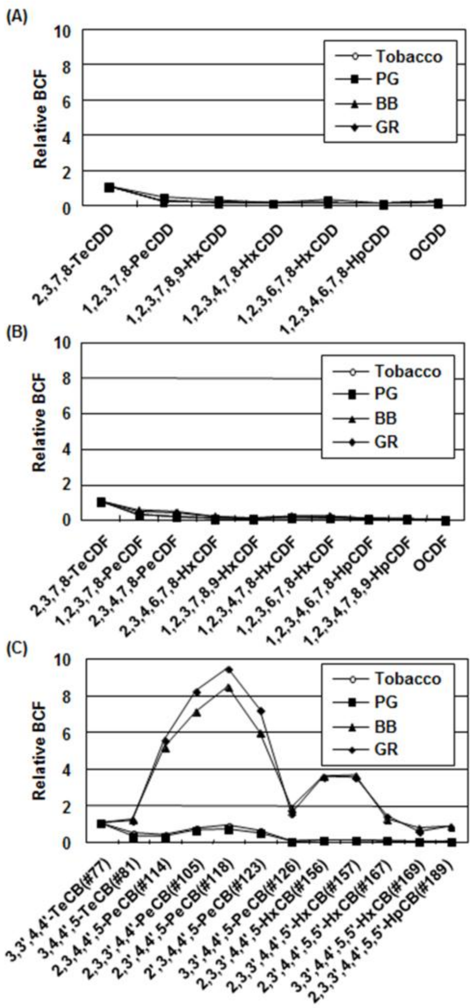

Figure 3 Relative BCFs of (A) PCDD, (B) PCDF, and (C) PCB congeners in tobacco plants and three *C. pepo* cultivars grown in soil contaminated with dioxins and dioxin-like compounds.
The relative BCF for each PCDD, PCDF, and PCB congener was calculated by dividing the BCF of the congener by the BCF of 2,3,7,8-TeCDD, 2,3,7,8-TeCDF, or 3,3',4,4'-TeCB, respectively (that is, the corresponding congener that accumulated to the highest concentration in tobacco).

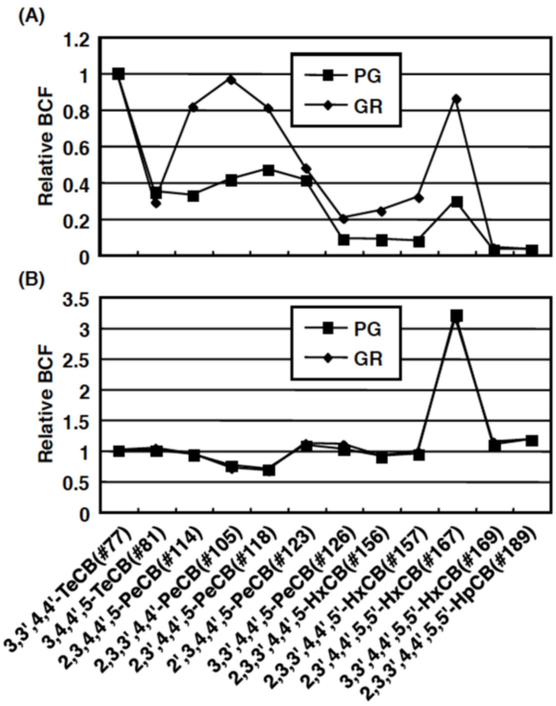

Figure 4 Relative BCFs of PCBs in (A) aerial parts and (B) roots of *C. pepo* cultivars PG and GR grown in hydroponic medium containing dioxins and dioxin-like compounds.
BCFs were calculated by dividing the concentration of each PCB in the aerial parts and roots of a plant by the corresponding concentration in soil. The relative BCF for each PCB congener was calculated by dividing the BCF of the congener by the BCF of 3,3',4,4'-TeCB.

independent of hydrophobicity. This efficient uptake of *ortho*-chlorinated congeners has also been observed in pumpkin[9]. The aerial parts of *C. pepo* plants grown hydroponically showed a PCB BCF pattern that was somewhat similar to the pattern for plants grown in soil (Figure 4A), but the BCF pattern in the roots was different[12] (Figure 4B). These results indicate that translocation from the roots to the aerial parts as well as desorption from soil was involved in the selective accumulation of *ortho*-chlorinated PCB congeners.

Hydrophobic compounds in plants are translocated via the xylem sap[2, 9], which contains various amino acids, inorganic elements, sugars, plant hormones, and proteins[15]. Recently, xylem sap proteins in *C. pepo* and cucumber were reported to bind and dissolve dieldrin[16]. These proteins may have been responsible for the selective uptake of PCB congeners observed in the current study.

We expect that our results will be useful for the development of efficient phytoremediation methods as well as for clarification of the mechanism of the uptake of hydrophobic compounds by members of the Cucurbitaceae.

Acknowledgements
This work was funded in part through a Grant-in-Aid for Scientific Research A from the Ministry of Education, Culture, Sports, Science and Technology of Japan (nos. 17208029 and 23241028).

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