Reduction of cadmium uptake of eggplant (*Solanum melongena*) by grafting onto *Solanum torvum* rootstock and characterization of cadmium translocation from roots to shoots

Tomohito Arao

National Institute for Agro-Environmental Sciences

Kannondai 3-1-3, Tsukuba, Ibaraki, 305-8604, Japan

arao@affrc.go.jp

Abstract: We determined cadmium (Cd) concentrations in eggplant grown on different rootstocks in Cd-polluted soil and unpolluted soil. Grafting onto *Solanum torvum* reduced eggplant fruit Cd concentrations by 63% to 74% in Cd-polluted soil and unpolluted soil compared with grafting onto *Solanum melongena* and *Solanum integrifolium*. Stem and leaf Cd concentrations of scions on *S. torvum* were about 30% of those on *S. integrifolium*, so Cd translocation from roots to shoots was apparently reduced in grafted plants on *S. torvum*. The Cd concentration of xylem sap in stems of *S. torvum* was 22% of that in stems of *S. melongena* in Cd-polluted soil. We examined the characteristics of Cd absorption in roots and Cd translocation from roots to shoots between *S. melongena* and *S. torvum* over 7 days using a hydroponic culture. Although there is no significant difference in Cd concentration in the roots of *S. melongena* and *S. torvum*, Cd concentration in the shoots and xylem sap was higher in *S. melongena* than in *S. torvum*. By evaluating symplastic Cd absorption in roots, using enriched isotopes ^{113}Cd and ^{114}Cd, and measuring the kinetics in xylem loading, we characterized Cd absorption and translocation for *S. torvum* and *S. melongena*. A concentration dependent study in roots indicated that Km values were almost the same for species, but the Vmax value was 1.5-fold higher in *S. melongena* than in *S. torvum*. A concentration dependent study in xylem loading indicated that Vmax was almost the same, but Km values were approximately 7-fold higher in *S. torvum* compared to *S. melongena*. These results, together, suggest that the affinity for Cd in the xylem loading process is a critical factor for determining the different Cd concentrations in the shoots between both plants under low Cd concentration conditions.

Keywords: Reduction of Cd, eggplant, *Solanum torvum*, grafting, xylem loading,

1. Introduction

Cadmium (Cd) is toxic to humans at concentrations lower than those at which it is toxic to plants, because its effects on humans are cumulative. A health-based guidance value for Cd of 7 μg kg^{-1} bodyweight per week [the provisional tolerable weekly intake (PTWI)] has been established by the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization and the World Health Organization. The Food Safety Commission Secretariat of Japan has set a PTWI of 7 μg kg^{-1} body weight per week for Cd. Recently, the European Food Safety Authority established a tolerable weekly intake for Cd of 2.5 μg kg^{-1} body weight. The weekly intake of Cd from foods in Japan in 2001 was estimated to be 4.1μg kg^{-1} body weight. A re-evaluation of Cd is scheduled for the 2010 meeting of the JECFA.

The CODEX Alimentarius Commission of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) proposed a new international standard for Cd concentrations in a variety of staple foodstuffs; for fruiting vegetables and roots vegetables, this level equals 0.05 and 0.10 mg Cd kg^{-1}, respectively. About 7% of 381 samples of eggplant (*Solanum melongena*), 22% of 165 samples of okra (*Abelmoschus esculentus*), and 10% of 302 samples of taro (*Colocasia esculenta*) contained Cd concentrations above these limits in a field and market basket study during 1998–2001 in Japan. Under these circumstances, we urgently need to develop technologies to suppress Cd absorption by crops.

The uptake of Cd by plants varies not only among plant species, but also among cultivars[1, 2]. Li et al. [3] reported significant variation in the grain Cd level of sunflower, durum wheat, and flax. Soybean seed Cd levels ranged from 0.46 to 2.7 mg kg^{-1} among 17 cultivars [4], and Cd levels in rice grain ranged from 0.89 to 4.4 mg kg^{-1} among 31 cultivars [5] in Cd-polluted soil.

Accumulation of large amounts of Cd in the root may limit the accumulation of Cd in above-ground portions of the plant. Sugiyama et al. [6] found clear differences among soybean cultivars in the influence of rootstock cultivars on shoot Cd concentration. For eggplant, grafting represents a useful tool to cope with problems of soilborne diseases. Rootstocks used in Japan are eggplants (*S. melongena*), related species, and interspecific hybrids. We compared the Cd concentrations in fruits of eggplant grafted onto *Solanum torvum*, *Solanum integrifolium*, and *S. melongena* rootstocks grown in unpolluted soil [7]. Cd concentrations of eggplant fruits were lowest on *S. torvum* rootstock.

To date, there has been intensive research on the physiological mechanisms involved in Cd absorption and translocation from roots to shoots in Cd-hyperaccumulators, such as *Thlaspi caerulescens* and *Arabidopsis halleri* [8-

12]. However, little is known about the characteristics of Cd absorption and translocation in low Cd concentration crop conditions, especially with regard to eggplant. In addition, the different physiological mechanisms of Cd accumulation in the shoots of *S. melongena* and *S. torvum* have not been elucidated.

The main objectives of the present study were to determine Cd and other metal concentrations in eggplant fruits grown on different rootstocks in Cd-polluted soil and to determine the differences in uptake, translocation and distribution of Cd among *S. torvum*, *S. melongena* and *S. integrifolium* grown either in soil or in nutrient culture [13]. We also investigated the physiological characteristics of Cd absorption in roots and xylem loading in *S. melongena* and *S. torvum* under low Cd concentration medium conditions. We found that the capacity for xylem loading may be a critical factor for Cd accumulation in shoots of *S. melongena* and *S. torvum* [14].

2. MATERIALS AND METHODS

Grafting

The rootstocks cv. Torubamubiga- (*S. torvum*) and Tonashimu (*S. torvum*) seeds were sown in unpolluted soil. About one month after sowing of Torubamubiga- and Tonashimu, the rootstocks Daitarou (*S. melongena*), Hiranasu (*S. integrifolium*) and the scion cv. Senryou2 (*S. melongena*) seeds were sown in unpolluted soil. Grafting was done about one month after sowing of Senryou2.

Pot experiments 1—grafted plants—Cd in fruits and tissues

Pot experiments with three replications were carried out in 2005 and 2006 in a greenhouse at ambient temperature (14-34°C, 2005, 18-34°C, 2006) under sunlight. Pots (1/2000 a) were filled with 10 kg of Cd-polluted Fluvisol (1M HCl extractable Cd 1.9 mg kg^{-1}). The soil was polluted from irrigation water that had passed through mines. Transplanting was done about one month after grafting, on 8 June 2005 and 22 June 2006. Four to nine fruits were harvested separately from each experimental pot during July-August. Stems and leaves were harvested in mid-August.

Pot experiments 2—self-rooted plants—Cd in tissues and xylem sap

Daitarou and Torubamubiga- were transplanted into 1/5000-a pots filled with 3 kg of Cd-polluted soil at 28 and 53 days after sowing, respectively. Forty days after transplanting (days 68 and 93), xylem sap was collected for about 1 h through silicone rubber tubes connected at the cut portions and diluted 10-fold with 1% HNO$_3$.

Field experiments—grafted plants—Cd in fruits

Senryou2 scions on Torubamubiga- or Hiranasu rootstocks were grown in unpolluted Andosol (1M HCl extractable Cd 0.29 mg kg^{-1}) under field conditions (2.5 m x 1.6 m plots) with three replications in Tsukuba, Japan. A basal application of fertilizer was supplied at 30 g m^{-1} of N, 13g m^{-1} of P, and 25 g m^{-1} of K in the form of compound fertilizer. Four grafted plants were transplanted to one plot on 22 June 2006. First fruit of each plant was harvested during July 2006.

Solution culture—grafted plants—Cd in tissues

The soil was washed from the grafted plant roots with water, and plants were transferred to a solution culture. Seven days after the transfer of plants to the nutrient solution, the Cd concentration was adjusted to 0.09 or 0.9 µmol L^{-1} with CdCl$_2$. Fourteen days after the addition of Cd, the stem and leaves of scions, stem of rootstocks, and roots were harvested. Before harvesting, roots were rinsed in running tap water for 2 min and in 18-MΩ water for 1 min.

Xylem loading experiment and xylem sap collection

Time course- and concentration-dependent experiments were conducted using seedlings precultured for 14 days to symplastic Cd uptake in roots. Symplastic Cd concentration in roots was evaluated by the method using stable isotopes ^{113}Cd and ^{114}Cd [15]. Time-course and concentration-dependent experiments in xylem loading process were conducted using seedlings precultured for 20 days. The seedlings precultured for 20 days were transferred to 500 mL pot containing nutrient solution containing 90nM Cd. In concentration dependent experiment, after the seedlings were transferred to 500 mL pot containing buffer solution containing 0-1200nM Cd for 8 hours, the xylem sap was collected as described below. Soft rubber tubes were fixed over decapitated stem after decapitating at approximately 1 cm above the roots and xylem sap was collected with a micropipette for 30 min. All experiments were conducted with three replicates.

Effect of a metabolic inhibitor on Cd absorption in roots and xylem loading

To investigate the effect of a metabolic inhibitor, seedlings of *S. melongena* and *S. torvum* were exposed to the nutrient solution (500mL, one seedling per pot) containing 90 nmol L^{-1} Cd with or without 0.5 µM carbonyl cyanide-m-chloro-phenyl-hydrazone (CCCP). CCCP in an ethanol solution was added to the nutrient solution, with a final ethanol concentration of 0.01% (v/v). The absorption experiment was conducted over 8 h, and xylem sap was collected as described above. Inhibitor experiment was conducted with three replicates.

Chemical analysis of plant tissues and statistical methods

Harvested leaves, stems, roots, and fruits were dried in an oven at 75°C for three days and ground to a fine powder. Half a gram of each sample was digested in 5 mL HNO$_3$:H$_2$O$_2$ (5:1, v/v) in a microwave oven (mls 1200, MILESTONE, FKV, Italy). Concentrations of Cd and other elements in digested samples and xylem sap were

determined by inductively coupled plasma – optical emission spectroscopy (Vista-PRO, Varian, Inc., Palo Alto CA,. USA). [113]Cd and [114]Cd in roots were analyzed using ICP-MS. Student's t-test and the least-significant difference (LSD) test were used to test for statistical significance in differences between treatments.

3. RESULTS AND DISCUSSION

In 2005, Cd concentrations of eggplant fruits growing on Torubamubiga- (*S. torvum*) and Daitarou (*S. melongena*) rootstocks in Cd-polluted soil were 0.13 and 0.50 mg [kg fw]$^{-1}$, respectively. In 2006, Cd concentrations of eggplant fruits growing on Torubamubiga-, Tonashimu (*S. torvum*), and Hiranasu (*S. integrifolium*) rootstocks in Cd-polluted soil were 0.15, 0.16, and 0.46 mg [kg fw]$^{-1}$, respectively. There were no significant differences in fruit concentrations of any metals except Cd. The average fresh weights of fruits were 82.6 g on Hiranasu, 86.0 g on Torubamubiga-, and 88.3 g on Tonashimu. In unpolluted soil, the Cd concentration of eggplant fruits growing on Torubamubiga- rootstock was 37% of the concentration of fruits growing on Hiranasu rootstock. Grafting onto *S. torvum* thus reduced eggplant Cd concentrations by 63% to 74%. There were no significant differences in fruit concentrations of any metals except Cd. There were no significant differences in average fresh weight of fruits between *S. torvum* and *S. integrifolium* rootstocks.

Stem and leaf Cd concentrations of scions on Torubamubiga- and Tonashimu rootstocks were about 30% of those on Hiranasu, so Cd translocation from roots to shoots was apparently reduced in grafted plants. K and Cu concentrations of stem and leaf of scions on Torubamubiga- were higher than those on Hiranasu, but no metal concentrations of stem and leaf were significantly lower on Hiranasu than on Torubamubiga-. So it is likely that the concentrations of Cd and other metals in the shoots of the scion are controlled by independent mechanisms.

Stem and leaf Cd concentrations of Torubamubiga- were also lower than those of Senryou2 (*S. melongena*) and Daitarou (*S. melongena*). There were no significant differences (P = 0.01) in any other stem and leaf metal concentrations between Daitarou and Senryou2 grown in polluted soil. The Cd concentration of xylem sap in stems of Torubamubiga- was 22% of that in stems of Daitarou. There were no significant differences in xylem sap concentrations of any metals except Cd.

In grafted plants grown in solution culture, the Cd concentrations of stem and leaves of scions and stem of rootstocks were also low when the rootstock was *S. torvum*, but the Cd concentration of roots of *S. torvum* was the same as that of *S. melongena*.

Florijn and van Beusichem [16] distinguished two groups of maize inbred lines: one with low shoot but high root Cd concentrations ('shoot Cd excluder'), and one with similar shoot and root Cd concentrations ('non-shoot Cd excluder'). Sugiyama et al. [6] reported that Cd retention in the roots of soybean is an important mechanism in regulating its translocation to the shoots and seeds. The Cd concentration of xylem sap in stems of Torubamubiga- was 22% of that in stems of Daitarou, so the reduced Cd translocation from root to shoot could be accounted for by differential loading of Cd into the xylem. Hart et al. [17] reported that movement of Cd through the root and into the xylem may have been the cause of differential Cd partitioning in two durum wheat isolines. The loading of Cd into xylem tissues for transport to shoots is a possible mechanism. Zn and Cd are translocated from root to shoot by P-type ATPase AtHMA4 in roots of Arabidopsis thaliana [18]. A similar transporter could be present in *S. torvum* and *S. melongena*, but the transporter of *S. torvum* might have less affinity for Cd than that of *S. melongena*.

The Cd concentration in xylem sap and shoots was increased with the increase of Cd concentration in the medium in both plants. But, those in *S. melongena* was higher than those in *S. torvum*. In the kinetics experiments evaluated by symplastic Cd uptake rate, although Km values were almost same in both plants, Vmax values were 1.5-fold higher in *S. melongena* than in *S. torvum*, suggesting uptake in both plants is mediated by a transporter that exhibits a similar affinity for Cd and the density of the Cd transporter in the root cell membranes is higher in *S. melongena* than in *S. torvum*. In xylem loading process, Km values were approximately 7-fold higher in *S. melongena* than in *S. torvum*. These results together suggest that xylem loading process is a critical factor for determining Cd accumulation in the shoots of both plants.

A metabolic inhibitor, carbonyl cyanide-m-chloro-phenyl-hydrazone (CCCP) inhibited Cd absorption and translocation from roots to shoots in both plants. This suggests that Cd absorption in roots and Cd translocation from roots to shoots via the xylem loading process, under low Cd concentration conditions, are partly mediated by an active energy-dependent process in both plants.

S. torvum develops noteworthy physiological mechanisms to suppress Cd concentrations in the shoots, compared to *S. melongena*. In this study, we clearly show that Cd absorption in roots and xylem loading was dependent on energy and a proteinous transporter. The differences seen in Cd-xylem loading capacity is mainly ascribed to the different Cd concentrations in the shoots between *S. melongena* and *S. torvum* under low Cd concentration conditions. Although the symplastic Cd absorption in roots is slightly higher in *S. melongena* than in *S. torvum*, the contribution of symplastic Cd absorption is assumed to be small in a low Cd concentration medium.

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