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**Title**

Characterization of Cd uptake in roots and translocation from roots to shoots in *Solanum melongena* and *Solanum torvum*

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## Introduction

Approximately 7% of 381 samples of eggplant (*Solanum melongena*) contained Cd concentrations above the limit adopted by the Codex Alimentarius Commission (0.05 mg Cd kg<sup>-1</sup> fw) in a field- and market-basket study during 1998-2001 in Japan (Ministry of Agriculture Forestry and Fisheries of Japan, 2002); despite the fact that these crops were cultivated in non-polluted fields. New technologies for reducing the Cd levels in eggplant are urgently required in Japan. Takeda et al. (2007) found that cadmium concentration in eggplant fruits could be drastically reduced by grafting with *Solanum torvum* rootstock. In addition, Arao et al. (2008) suggested that differential symplastic Cd uptake and xylem loading process might also account for the difference in Cd translocation to the stem of rootstocks and the stem and leaves of scions. However, it is still unclear why a grafting of *S. torvum* can reduce Cd accumulation in the shoots of *S. melongena*. We thus examined characteristics of Cd uptake in roots and translocation from roots to shoots via xylem loading process in both plants. Additionally, we examined effect of other element concentration in roots and xylem sap with the increase of Cd concentration in the medium.

## Materials and methods

Seeds of *S. melongena* cv. Sennryou 2 and *S. torvum* cv. Torubamubiga- were germinated and grown on moist perlite for one and two months, respectively. The seedlings were then transferred to 30-L containers containing Enshi standard nutrient solution as described in the previous paper (Mori et al. 2009) and were grown for 10-20 days. The nutrient solution was changed every 7 days. The pH of nutrient solution was adjusted at 5.5 using 0.1 mol L<sup>-1</sup> NaOH solution. Growth conditions for preculture were controlled with temperature at 25°C and relative humidity at 60-80% with natural lights. After preculture, each experiment was conducted.

In the long term experiment, seedlings precultured for 10 days described above were grown for 7 days in each 2-L plastic container containing the nutrient solution supplemented with 0, 90 and 900 nmol L<sup>-1</sup> Cd. Concentration dependent (0-1200 nM) experiment, involving <sup>113</sup>Cd- uptake in roots, was conducted over a period of 0.5 h, using seedlings precultured for 14 days. Symplastic <sup>113</sup>Cd concentration in roots was evaluated using the stable isotopes <sup>113</sup>Cd and <sup>114</sup>Cd (Mori et al. 2009). In the time course dependent experiments involving xylem loading process, the seedlings precultured for 20 days were each transferred to 500 mL plastic bottles containing the nutrient solution with 90 nmol L<sup>-1</sup> Cd. Xylem sap was collected at 0-48 hours. In the concentration dependent experiment, the seedlings were each transferred to 500 mL plastic bottles containing buffer solution (2 mmol L<sup>-1</sup> MES-Tris, 0.5 mmol L<sup>-1</sup> CaCl<sub>2</sub>, pH 6.0) with various concentrations of Cd (0-1200 nmol L<sup>-1</sup>). After 8 hours, the xylem sap was collected as described below. Soft rubber tubes were fixed over the decapitated stems after decapitating at approximately 1 cm above the roots. Xylem sap was collected with a micropipette for 30 min. All experiments were conducted with three replicates. Concentrations of Cd and other elements in digested samples were determined by inductively coupled plasma optical emission spectroscopy (ICP). <sup>113</sup>Cd and <sup>114</sup>Cd in roots were analyzed using ICP-mass spectroscopy (MS).

## Results and Discussion

In the long term experiment for 7 days, Cd concentrations in roots, xylem sap and shoots of *S. melongena* and *S. torvum* increased with the increase of Cd concentration in the medium. Although there was no difference of Cd concentration in the roots of *S. melongena* and *S. torvum* grown under the nutrient solution containing 90 nmol L<sup>-1</sup> Cd, Cd concentrations in xylem sap and shoots of *S. melongena* were higher than those of *S. torvum*, corresponding with the previous reports (Arao et al. 2008, Mori et al. in press). These results suggest that *S. torvum* has developed some physiological mechanisms to suppress Cd translocation from roots to shoots, compared with *S. melongena*. We then characterized the symplastic Cd uptake rate in roots using the stable isotopes <sup>113</sup>Cd and <sup>114</sup>Cd. A kinetics study showed that although K<sub>m</sub> values were almost the same in both plants, V<sub>max</sub> values were 1.5-fold higher in *S. melongena* than in *S. torvum*, suggesting Cd uptake in roots of 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*. A kinetics study in xylem loading process revealed that K<sub>m</sub> values were approximately 7-fold higher in *S. torvum* than in *S. melongena*. These results together suggest that xylem loading process is a critical factor for determining Cd accumulation in the shoots of both plants grown under a low Cd concentration in the medium. In the long term uptake experiment, some elements such as Zn, Fe, Mn and Ca in roots and xylem sap of both plants tend to decrease with the increase of Cd concentration in the medium, suggesting that the reduction of these elements' concentration in roots might be ascribed to competition between these elements and Cd at uptake sites in roots. Therefore, we suggest that Cd uptake and translocation from roots and shoots in both plants are partly mediated by transporters involving the transport of these elements. Further investigation is required to clarify the candidate mechanism of Cd uptake and translocation from roots to shoots in both plants.

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