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## **Title**

Differences among soybean cultivars with regard to the cadmium-accumulation patterns in various organs

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#### **Introduction**

In Japan soybean is an important traditional food, and sauce and paste made from it are intrinsic to the Japanese gastronomic culture. The Ministry of Agriculture, Forestry and Fishers, Japan, conducted a large-scale survey of domestic agricultural products. The results revealed that the cadmium (Cd) concentration in one-sixth of the total soybean produce exceeded 0.2 mg kg<sup>-1</sup>, the international standard proposed by the Codex Alimentarius Commission. Further, the soybean crops had a considerably higher Cd content than other field crops (MAFFJ, 2002). Moreover, a survey of 6 crops cultivated in the main producing districts of the United States (Wolnik et al., 1983) and a comparative study of crops grown under identical soil conditions (Bingham et al., 1975, MacLean, 1976) both revealed that soybean has higher Cd concentrations than cereals. These results indicate that soybean absorbs Cd more easily than do other crops.

Sugiyama et al. (2005) investigated 150 soybean varieties that are among the popular cultivars and breeding lines in Japan; on the basis of their results, the soybean varieties were separated into 2 groups. In one group, the young shoots exhibited excessive Cd uptake and, accordingly, the seeds of these plants had high Cd concentrations. A grafting experiment proved that the Cd concentration in seeds of scion cultivars was determined mainly by the rootstock cultivars, i.e., the Cd-accumulation capacity of the roots (Sugiyama et al., 2007). However, the scion cultivars also influenced the seed Cd concentration. The objective of our study is to demonstrate how Cd is translocated from the root to the shoot and accumulates in each shoot organ during soybean development.

#### **Materials and Method**

We investigated the soybean cultivars Suzuyutaka, Hatayutaka, Enrei, and Kanto 100 and found that the seed Cd concentration in the cultivars decreased in the order in which they have been named (Arao et al., 2003). The experiment was conducted using 3.7-L pots, each filled with 3.0 kg of air-dried Cd-polluted soil (paddy field Andosol; 0.1 M HCl-extractable Cd, 3.7 mg  $kg^{-1}$ ) that had been polluted by irrigation with water that had flowed through mines. A basal fertilizer containing 0.06 g N, 0.6 g P<sub>2</sub>O<sub>5</sub>, and 0.3 g K<sub>2</sub>O in the form of ammonium sulfate, superphosphate of lime, and potassium sulfate, respectively, was applied to each pot. The soybeans were grown under sunlight in a greenhouse at ambient temperature (18–30 °C). Shoots were harvested at 10 stages: the first-node stage (V1), third-node stage (V3), fifth-node stage (V5), full-bloom stage (R2), pod-formation stage (R3), full-pod stage (R4), seed-formation stage (R5), mature-seed stage (R6), early-maturity stage (R7), and full-maturity stage (R8). Plants at each stage were grown in 4 pots. Shoots were separated into stems, leaves, petioles, pods, and seeds. Fallen leaves were collected twice every day. The shoots of Suzuyutaka and Enrei were divided at every node on the main stem, and the organs within each internode were harvested from R3 onward. All the organs were immediately washed with deionized water to remove the adherent soil. Their dry weight and Cd concentrations were then measured.

### **Results and Conclusion**

At R8, the dry weight of the Suzuyutaka shoots was 70 g pot<sup>-1</sup> and that of the Kantou 100 shoots was 90  $g$  pot<sup>-1</sup>. During development, the Cd concentrations in the shoots and all the excised organs were higher in Suzuyutaka than in Kantou 100. At R8, the Cd concentration in the Suzuyutaka shoots was 5.7 mg pot<sup>-1</sup> and greatly differed from that in the Kanto 100 shoots (2.1) mg pot<sup>-1</sup>). The dilution effect due to the biomass cannot explain this difference. In both cultivars,

the Cd concentration was higher in the vegetative organs, i.e., the leaves, petioles, and fallen leaves, than in the other organs. This concentration was especially high in the fallen leaves collected during the early reproductive stages (Fig. 1). Older leaves on the lower nodes of the main stem fell earlier than younger leaves. The fallen leaves collected at R4 were leaves and petioles from nodes 1, 2, and 3 of the main stem. In Suzuyutaka, the average Cd concentration in the leaves collected from nodes 1, 2, and 3 of the main stem at R3 was 41 mg  $kg^{-1}$  and was almost equal to that in the fallen leaves collected at R4  $(46 \text{ mg kg}^{-1})$ . Further, newer leaves grown on the upper nodes of the main stem had lower Cd concentrations (average, 9.9 mg  $kg^{-1}$ ; leaves were present on the uppermost node counted node 9). During development, the average Cd concentration in the fallen leaves decreased because the leaves that grew on the middle and upper nodes and had low Cd concentrations fell in rapid succession. In Enrei, similar results were observed with regard to the Cd concentration in the leaves.



Figure 1. Cadmium concentrations in shoot organs during soybean development.

The dry weight of the whole leaves (including petioles and fallen leaves) stopped increasing after R5. At R5, the Cd concentration in the whole leaves was  $0.29 \text{ mg pot}^{-1}$  in the case of Suzuyutaka and  $0.11$  mg pot<sup>-1</sup> in the case of Kantou; these concentrations were almost identical to those recorded at R8 (Suzuyutaka,  $0.27 \text{ mg pot}^{-1}$ ; Kantou 100, 0.11 mg pot<sup>-1</sup>). The total concentration of Cd distributed from the shoots to the whole leaves was 67% in the case of Suzuyutaka and 57% in the case of Kantou 100. After R5, the Cd concentration in newly developed pods and seeds, cumulatively increase with the dry weight. At R8, the concentration of Cd distributed from the shoots to the seeds was 13% in the case of Suzuyutaka and 21% in the case of Kantou 100. These results suggest that cultivars with a low capacity of Cd accumulation in the roots exhibit a mechanism that prevents Cd accumulation in the seeds by promoting its accumulation in the leaves. We believe that this mechanism serves to eliminate excess Cd from the plants through falling leaves.

Next, we compared cultivars that had high Cd concentrations. In Hatayutaka (seed Cd

concentration, 1.10 mg  $kg^{-1}$ ), the whole-leaf to shoot ratio of the Cd concentration was 2.8 (10.2) mg kg<sup>-1</sup>/3.7 mg kg<sup>-1</sup>), and was higher than in Suzuyutaka (2.3, i.e. 13.3 mg kg<sup>-1</sup>/5.7 mg kg<sup>-1</sup>). In a previous grafting experiment performed using Hatayutaka, the seed Cd concentration was found to be influenced by the scion cultivar (Sugiyama et al., 2007). This suggests that Hatayutaka can efficiently eliminate Cd via its accumulation in the leaves.

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