

# **Alteration of Cesium Behavior and Gene Expressions of Alkali Metal Transporters Induced by Short Day Transition in Model Tree, Poplar**

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## Summary

### Abstract

Woody plants which constitute the major part of forest ecosystem were contaminated by radionuclides released from Fukushima Dai-ichi Nuclear Power Plant (FDNPP) accident in March 2011. Radioactive cesium (Cs) concentration in wild plants including woody plants exhibited still high in the forest area. Therefore, to identify the regulatory factor for Cs behavior, gene-based approach was attempted with poplar Hybrid aspen T89 (*P. tremula* x *P. tremuloides*). Investigation of the genes related to Cs transport by combining Cs behavior and the gene expressions of potassium (K) transporters was performed in woody plant.

In Part I, regulations of  $^{42}\text{K}$  and  $^{137}\text{Cs}$  uptake with short day (SD) transition were investigated. During the shift from long day (LD) to SD,  $^{42}\text{K}$  accumulation to shoot organs was kept constant and the genes encoding several K transporters, KUP/HAK/KT family and VICCs channels, exhibited almost stable expression levels. In contrast,  $^{137}\text{Cs}$  accumulation in shoot was significantly down-regulated under SD6 (SD treatment for 6 weeks) condition. These results showed that  $^{137}\text{Cs}$  uptake and transport were

suppressed in dormancy induced poplar, suggesting Cs uptake under SD condition would be independent to K transport.

In Part II, change of Cs behavior through foliar absorption with SD transition was investigated. In foliar application, applied  $^{137}\text{Cs}$  was transported to the organs lower than applied leaf. On the other hand, under SD condition, the transfer of  $^{137}\text{Cs}$  was switched to the upper organs. In the nodes, the gene encoding putative K xylem loading channel, SKOR-like, was highly induced at SD6 condition. The analysis using micro-autoradiography technique showed  $^{137}\text{Cs}$  transport thorough foliar application was mainly regulated by phloem transport under LD condition and by xylem and phloem transport under SD condition. In conclusion, this study presents the novel K/Cs transport mechanism combining xylem and phloem transport in tree.

## **Introduction**

In 2011, the FDNPP accident released a large amount of radionuclides into the environment. Due to its long half-life, radioactive cesium ( $^{137}\text{Cs}$ ) was considered one of the main contaminant substances. Cesium is not an essential nutrient for plant growth, but it is considered that Cs is taken up

by K transporters due to their similarities of chemical property. In the field environment, it was also reported that radioactive compounds penetrate and/or diffuse into the tree body through the surface deposition. Because of these contamination processes, woody plants still kept high  $^{137}\text{Cs}$  within their body.

In this research, I focused on short day (SD) transition as one of the seasonal variations in tree life cycle. In tree, SD transition induced translocation of internal elements for nutrients storage in various organs. Mineral distribution was significantly altered under SD condition, thus, SD transition is considered as the drastic event for the shift of nutrient balance in tree. Poplar can be grown under shorted annual environmental cycle in lab-scale experiment and its genome information was already opened. For investigating the Cs behavior and the molecular mechanisms related to Cs transport in tree, I focused on the SD transition process in poplar Hybrid aspen T89 (*P. tremula* x *P. tremuloides*).

## Part I

Cesium uptake and translocation within the plant body are thought to be mediated by  $K^+$  transport systems. I investigated  $^{137}Cs$  and  $^{42}K$  localizations using a model tree, poplar, under LD and SD conditions. Poplar is a perennial deciduous tree with a characteristic seasonal cycle of growth and dormancy. Woody plants shift their growth stage when they perceive changes in photoperiod and temperature. Japanese native poplar, *P. maximowiczii* showed a decrease in leaf K concentration following dormant bud formation. And the increase in K concentration in xylem sap was observed during the winter season in field-grown *P. nigra*. These behaviors imply the existence of re-translocation mechanisms for K. Potassium is transported through various systems within the plant body. Potassium ion uptake by the root is mediated by the KUP/HAK/KT transporter family and non-selective cation transport mechanisms such as voltage-independent cation channels. Based on these knowledges, I investigated the relationship between the change in K localization induced by SD and the behavior of Cs absorbed from the rhizosphere.

### **Shoot $^{137}\text{Cs}$ amount was down-regulated under SD conditions**

The uptake of Cs via root and its behavior within the plant body in LD and SD conditions were compared. To measure seasonal variations in Cs uptake, a  $^{137}\text{Cs}$  solution was added to the growth media under LD3 (LD treatment for 3 weeks, = SD0), LD9 (LD treatment for 9 weeks), and SD6 (SD treatment for 6 weeks after LD3) conditions. In the LD3 plants,  $^{137}\text{Cs}$  was localized entirely and the radioactivity level was highest in the apex. The LD9 plants showed the same  $^{137}\text{Cs}$  behavior as the LD3 plants. In the SD6 plants,  $^{137}\text{Cs}$  was localized mainly in the stem and root and the total  $^{137}\text{Cs}$  amount was lower than LD3 and LD9 plants. On the other hand, the amount of  $^{137}\text{Cs}$  in the roots was similar for all three conditions.

### **Potassium-42 uptake was constant under LD and SD conditions**

Poplar roots were treated with exogenous  $^{42}\text{K}$  and the amounts of  $^{42}\text{K}$  in the shoots and roots under LD3, SD2 (SD treatment for 2 weeks after LD3), SD4 (SD treatment for 4 weeks after LD3), and SD6 conditions were measured. Among these four conditions, no difference was found in the amount of  $^{42}\text{K}$  in the roots. This result implied that the transporter responsible for  $\text{Cs}^+$  uptake

in poplar might have limited involvement in  $K^+$  uptake since no decrease in K accumulation was observed when Cs accumulation was low.

### ***PttHAK-like1* was up-regulated by transition to SD**

I investigated the expression patterns of some  $K^+$  transporter under SD conditions. During the transition to the SD conditions, the expression of *PtKUP1* did not change significantly. *PttHAK-like1* showed steady expression until the transition to SD4 conditions and was up-regulated by about 1.5-fold under SD6 conditions. *PttHAK-like2* expression tended to decrease in SD2 and SD4 plants but maintained statistically steady-state transcription levels through SD transition. The expressions of *PttCNGC1-like1*, *PttCNGC1-like2* were also relatively constant under SD conditions.

Despite the steady expression of focused genes and constant  $^{42}K$  uptake activity through seasonal transitions, Cs accumulation activity was down-regulated under SD6 conditions. Therefore, the  $Cs^+$  and  $K^+$  transport systems are probably regulated separately in poplar.

## **Part II**

The transfer process of  $^{137}\text{Cs}$  within the forest ecosystem is time-consuming comparing to the other area, therefore, the behavior of  $^{137}\text{Cs}$  in woody plant is important to estimate the impact of  $^{137}\text{Cs}$  to the ecosystem. Poplar is perennial woody plant and has become a model plant for tree since full *P. trichocarpa* genomes are revealed. Here, I investigated the gene expressions in annual environmental cycle and Cs behavior with seasonal change to identify the Cs transport regulation system.

### **Two different transport pathways in hybrid poplar**

Root absorption is the main pathway for plant elements uptake and is directly involved in water-soluble  $^{137}\text{Cs}$  to be incorporated into plants after the fallout. Furthermore, foliar absorption of  $^{137}\text{Cs}$  deposited onto developed leaves is also concerned as another incorporating pathway for plants. To monitor the detailed Cs translocation mechanism through these two different-absorption pathways in poplar, I employed autoradiography technique. In root application, absorbed  $^{137}\text{Cs}$  was accumulated firstly in shoot apex and upper leaves. On the other hand, in the foliar application,



absorbed  $^{137}\text{Cs}$  was mainly detected in the applied leaf and the stem downward from the applied leaf. I also observed  $^{42}\text{K}$  behavior in root and foliar application. As a result, behaviors of  $^{42}\text{K}$  in plant with root and foliar application were similar to that of  $^{137}\text{Cs}$ .

### **Induction of *PttSKOR-like2* expression by SD and/or low temperature**

To examine the gene expression patterns in seasonal alteration, I employed the artificial annual environmental cycle. This growth system simulates seasonal conditions, spring, summer, fall and winter. Each condition is consisted with the combination of photo period and temperature conditions. The poplar shifted its growth conditions from summer to fall formed a dormant bud. After formation of dormant bud, poplar stopped its growth and, after the 4 weeks low-temperature treatment, poplar started to grow again under the LD condition.

I focused on  $\text{K}^+$  loading channel named as SKOR. SKOR is identified in *Arabidopsis thaliana*, and is known to have a transport activity of Cs. I identified two homologs in hybrid aspen, *PttSKOR-like1* and *PttSKOR-like2*, and the expression levels were compared by qRT-PCR. In SD0 condition, at

the start of artificial annual environmental cycle, expression level of *PttSKOR-like2* was about 18 times higher than that in *PttSKOR-like1* in root. In upper shoot, *PttSKOR-like2* was expressed similar to root, however, *PttSKOR-like1* expression was much lower. *PttSKOR-like2* expression was up-regulated gradually under SD condition and, at SD8 (SD treatment for 8 weeks after LD3), reached about 4-fold of that at SD0. *PttSKOR-like2* expression was temporary decreased at the onset of low-temperature condition but was up-regulated again. These indicate that the *PttSKOR-like2* expression in upper shoot and root is regulated by the environmental factors such as day length and/or temperature.

#### **Down-regulation of *PttSKOR-like2* expression under LD extended condition**

In artificial annual environmental cycle, *PttSKOR-like2* was up-regulated by SD transition. To identify inducing factor of *PttSKOR-like2*, poplar plants were treated in LD extended condition. *PttSKOR-like2* expression was decreased until LD9 condition, while was increased again from LD11 (LD treatment for 11 weeks) condition in root. In upper shoot, expression pattern was similar to that in root. Expression level was down-regulated until LD11

condition, but was increased again in LD13 (LD treatment for 13 weeks) condition in upper shoot.

### **Alteration of cesium behavior under different *PttSKOR-like2* expressed condition**

From the results of *PttSKOR-like2* expression patterns at artificial annual environmental cycle and LD extended condition, it was revealed that there were two different *PttSKOR-like2* expression patterns under different light conditions. To observe the correlation between Cs behavior and *PttSKOR-like2* expression pattern,  $^{137}\text{Cs}$  behavior was measured under different *PttSKOR-like2* expression condition. In foliar application, treatments under LD conditions (LD3 and LD9) resulted in the similar behavior that absorbed  $^{137}\text{Cs}$  was mainly detected in the applied leaf and the stem lower than the applied leaf. By contrast,  $^{137}\text{Cs}$  behavior was shifted to upper organs under SD condition.

### **Change of *PttSKOR-like2* expression with SD transition in node**

Cesium-137 behavior through foliar application was switched in node of

applied leaf. I focused on *PttSKOR-like2* expression patterns in node. Node tissues were harvested from plants at LD3 and SD6 and the induction of *PttSKOR-like2* expression was evaluated by qRT-PCR. In nodes, *PttSKOR-like2* was highly up-regulated under SD6 condition.

### **Spatial *PttSKOR-like2* expression profile with SD transition in node**

To determine spatial *PttSKOR-like2* localization, I carried out *in situ* hybridization in poplar. In LD3 condition, *PttSKOR-like2* was localized between xylem and phloem around the node. In SD6 condition, *PttSKOR-like2* was also localized adjacent to xylem, in particular, between phloem and xylem in node. Therefore, it was considered that *PttSKOR-like2* would regulate Cs transport from phloem to xylem through SD transition.

### **Different <sup>137</sup>Cs localization under LD3 and SD6 condition in node**

I performed micro-autoradiography which can show the distribution of radionuclides at the tissue/cellular levels in plants. Under LD3 condition, <sup>137</sup>Cs absorbed through foliar application was located at the phloem and xylem of lower stem, and at the xylem of upper stem. Under SD6 condition,

absorbed  $^{137}\text{Cs}$  was mainly detected in upper stem. The  $^{137}\text{Cs}$  signal at the phloem and xylem of upper stem was much higher than that at lower stem, suggesting that absorbed  $^{137}\text{Cs}$  through foliar absorption under SD6 condition would be transported to the upper part through the pathway of phloem to xylem. Because *PttSKOR-like2* was strongly up-regulated in node under SD6 condition, it was strongly suggested that PttSKOR-like2 transport Cs/K from phloem to xylem around the node.

#### **Change of internal $^{137}\text{Cs}$ accumulation with SD transition**

Cesium-137 was applied to culture medium at LD3 and continued to grow for additional 6 weeks under LD and SD conditions. Under LD9 condition,  $^{137}\text{Cs}$  absorbed from the root was localized to the whole poplar body. In the SD6 plants, similar to the result described in Part I,  $^{137}\text{Cs}$  accumulation in leaves seemed to be decreased compared to LD treatment. Moreover,  $^{137}\text{Cs}$  was transported preferentially toward upper organs under SD6 condition. In this regulation, PttSKOR-like2 would have important role in nodes for controlling Cs/K allocation with SD transition.

## **Discussion**

Among the various environmental conditions, K transport activity in the SD transition process was not well investigated. The SD transition is the mainly focused in the study of dormancy initiation in woody plants, therefore, the approach of mineral nutrition was not well attempted. However, after the FDNPP accident, the behavior of Cs within the tree body becomes one of the main topics in the understanding the radionuclides distribution within the forest ecosystem. Based on the previous studies related to the K localization under SD condition, K and Cs accumulation activity was investigated in poplar. The K uptake from the rhizosphere was constant during the SD transition. In addition to that, for shifting from LD to SD conditions, expressions of several K uptake transporters also showed constitutive which is consistent with the constant K accumulation pattern during SD transition. In spite of these stable K uptake and transfer, Cs accumulation was significantly suppressed under SD6 condition. Not all genes encoding K transporters in poplar genome were examined in its expression, but this inconsistency between K and Cs uptake suggests that the existence of specifically Cs permeable transporter(s) which expression or activity are

down-regulated under SD condition.

It is known that SD transition induces the alteration in nutrient allocation among the plant organs. Indeed, SD transition caused the decrease of Cs uptake from the root and changes of Cs behavior in the foliar absorbed Cs. Related to these alterations of Cs behavior in SD transition, *PttSKOR-like2* was identified. *PttSKOR-like2* was highly up-regulated in SD node and expressed between xylem and phloem. Moreover, it was shown that Cs was accumulated preferentially into shoot apex, upper leaves and stem under SD condition. These SD-induced changes of Cs distributions indicate that Cs translocation activity from source to sink organs (from leaves to other) was shifted by SD transition. The spatial and temporal regulations of *PttSKOR-like2* expression suggest its important role in Cs translocation with SD transition.

Generally, woody plants keep high  $^{137}\text{Cs}$  concentration in their body during a relatively long period. Based on the identifications shown here, it is considered that the regulation of *SKOR* gene expression in tree may be involved in, at least in part, the long retention of  $^{137}\text{Cs}$  in forest ecosystem. Although the analysis of *PttSKOR-like2* in the transport activity for alkali

metal cations and the observation of Cs behavior using transgenic plant will be required for further evaluation, I hope, in the near future, these results might support the prediction for the  $^{137}\text{Cs}$  behavior in forest ecosystem and effective decontamination of woody plants.