岩手大学
IWATE UNIVERSITY

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Iwate University

The long-sought potassium-independent cesium transporters have been identified

In collaboration with National University corporation Shimane University and The University of Tokyo, Iwate University, for the first time in the world, discovered the potassium-independent cesium transporter and have shown the feasibility of cleaning up the contaminated soil using phytoremediation technique. The research group includes Dr. Takashi Akihiro, Faculty of Life and Environmental Science, Shimane University, Dr. Keitaro Tanoi of Graduate School of Agricultural Life Sciences, University of Tokyo, Dr. Mohammad Arif Ashraf, United Graduate School of Agricultural Sciences, Iwate University, Keita Ito, Graduate School of Integrated Arts and Sciences, Iwate University, Sayaka Kumagai Faculty of Agriculture, Dept. of Plant Bio Sciences, Iwate University and led by Dr. Abidur Rahman, Faculty of Agriculture and Next generation Agri Innovation Center, Iwate University.

Radioactive cesium accumulates in the soil in a nuclear accident and causes serious soil pollution problems. Soil is also polluted with other toxic metals such as cadmium and arsenic. In search of a solution to clean up the soils, this research group focused on a method called phytoremediation. This method uses plants (phytochemicals) to absorb harmful substances in the soil and purify the soil (remediation). Previous findings indicate that cesium uses potassium transporters to be absorbed in the plant. Since potassium is an essential element, it is impractical to use potassium transporter overexpressors to absorb sufficient cesium from soil, as the soil will be depleted of potassium, resulting in an environment where no plants will be able to grow. In this study, the group discovered for the first time potassium-independent cesium uptake proteins and its causative gene. This work demonstrates the possibility that plants can absorb radioactive cesium efficiently without depleting soil potassium, and open to the realization of a complete phytoremediation method for radioactive cesium contaminated soil that has not been established so far.

The results of this research will be published in the scientific journal "Molecular Plant" published by Cell Press on February 13, 2021 (JST).

Highlights of the research

(1) Discovered ABCG33 and ABCG37, which are potassium-independent cesium uptake carriers.

(2) Demonstrated the functionality of ABCG33 and ABCG37 in cesium uptake using yeast system. Radioactive cesium uptake was increased when ABCG33 and ABCG37 are expressed in yeast.

(3) It is expected that plants overexpressing ABCG33 and ABCG37 can be used to realize the phytoremediation method to clean up the soil contaminated with radioactive cesium.

1. Background

Soil pollution by toxic metals is a serious problem for agriculture. These toxic substances are absorbed by the plants from the soil and contaminate our food chain and cause various diseases. Radioactive cesium is one of these toxic metals, which is generated by the nuclear accident. Cesium is closely associated with potassium and previous studies demonstrated that cesium can be transported from the soil to the plant by the potassium transporters. Various techniques are in use to solve the cesium contaminated soil pollution problem. For example, methods such as scraping the soil surface and spraying a large amount of potassium on the soil to prevent plants from absorbing cesium were carried out. However, these methods have problems in terms of major changes in the soil environment and economic costs, so they have never been effective methods. Therefore, there was a high demand for the development of new methods for purifying harmful substances in the soil.

Iwate University established the "Iwate University Next Generation Agri-Innovation Research Center" in April 2018 to conduct research and development aimed at next-generation agricultural innovation. A research group led by Associate Professor Dr. Rahman, a faculty member of the Faculty of Agriculture Iwate university and a member of agri-innovation center, focused on a method called phytoremediation to purify soil contaminated with radioactive cesium. This is a method of purifying (remediating) soil pollution by absorbing and accumulating harmful substances in plants while growing a specific plant (phytoremediation) in a soil-contaminated area and removing the plant (Note1). The advantage of this method is that, unlike the conventional method, contaminated soil can be purified without significantly changing the soil environment (Fig. 1). However, developing this method possesses many challenges. Especially for cesium, as it is also transported by potassium transporters. If the phytoremediation approach is taken to clean up the cesium contaminated soil by using plants overexpressing potassium transporters, those plants will also absorb a huge amount of potassium,

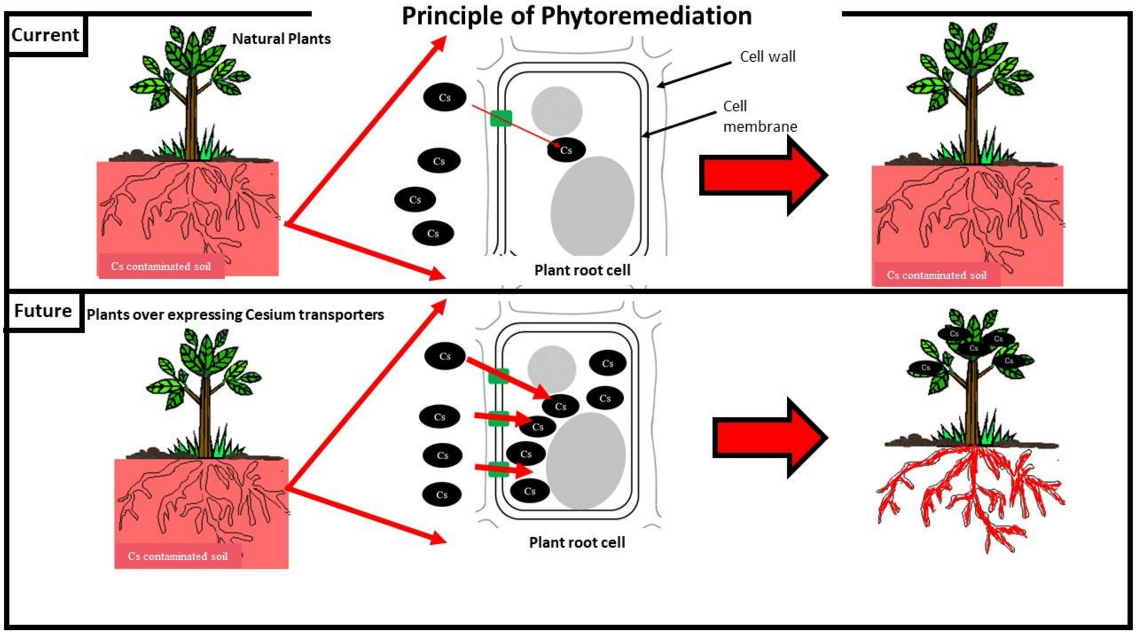

Figure 1. Phytoremediation is a green eco-friendly technique where plants are used to absorb the soil contaminants. In order to use the technique for cesium, potassium-independent cesium transporter must have to be found (shown in green symbols)

resulting in a potassium depleted land, where farming would not be possible. The research group focused on discovering cesium transport proteins that function independently of potassium.

2. Research content

In quest of search for new cesium transporters, this study focused on the ABC protein (Note 2), which has been shown to transport various toxic metals such as cadmium, arsenic, lead and mercury in earlier studies. Screening (Note 4) was performed at a concentration of 1.5 mM cesium using *Arabidopsis thaliana* (Note 3) mutants lacking the ABC proteins. From the screen, the *abcg33abcg37*double-deficient mutants showed resistance to cesium (Fig. 2). This result suggests that ABCG33 and ABCG37 are potential cesium transporter proteins which act redundantly.

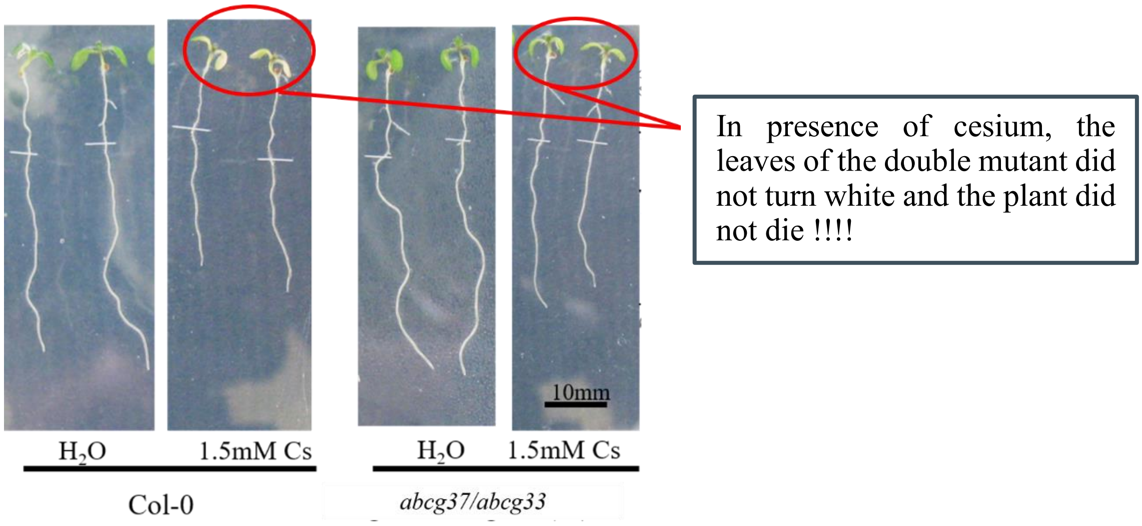

Figure 2. The response of double mutant *abcg33abcg37* to cesium

To confirm that these ABC proteins transport cesium, in collaboration with Dr. Keitaro Tanoi of The University of Tokyo, the group next assessed the uptake of cesium using radioactive cesium (^{137}Cs). Compared with the wild-type control plants, the double mutant accumulates much less cesium in their root in the presence or absence of potassium (Figure 3), indicating that ABCG33 and ABCG37 are required for Cesium transport and loss of these proteins results in less Cs transport. The next question to address is whether these proteins transport potassium. To address this, the plants' growth response in the presence or absence of potassium was assessed. The double mutant growth response did not differ from the wild-type control plants, confirming that ABCG33 and ABCG37 do not transport potassium.

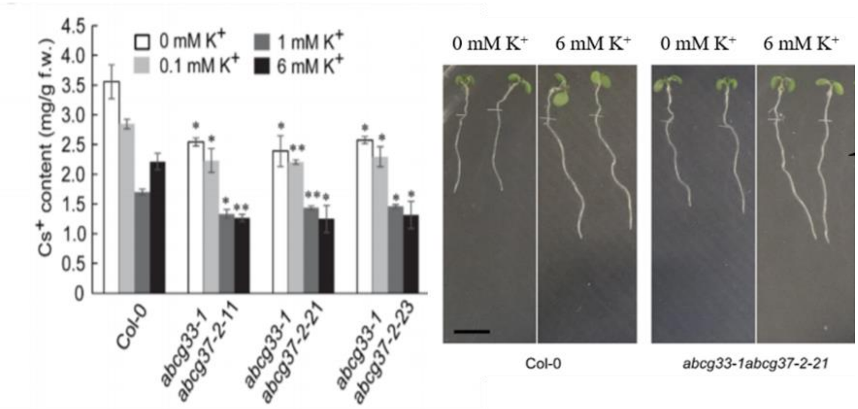

Figure 3. The response of double mutant *abcg33abcg37* to cesium transport and potassium

From the results so far, ABCG33 and ABCG37 can be considered to be potassium-independent cesium uptake proteins. To confirm the cesium transport capacity of ABCG33 and ABCG37, in collaboration with Dr. Takashi Akihiro of Shimane University, the group used yeast system. ABCG33 and ABCG37 were expressed in yeast (*Saccharomyces cerevisiae*) and radioactive cesium absorption experiment was conducted. The amount of radioactive cesium absorbed by yeast expressing ABCG33 and ABCG37 was higher than that of the control group (Fig. 4).

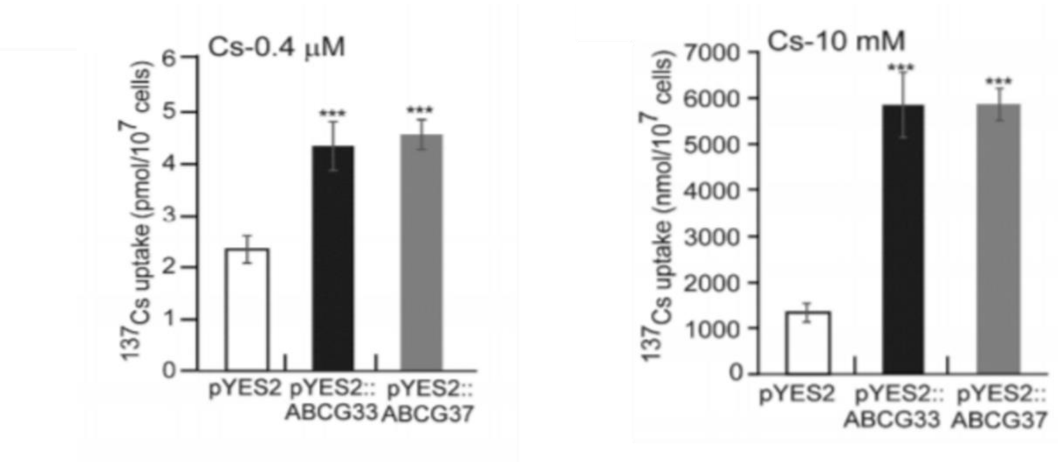

Figure 4. ABCG33 and ABCG37 uptake cesium in yeast system

Furthermore, ABCG33 and ABCG37 and *Arabidopsis thaliana* high-affinity potassium transporter (AtAKT1) were expressed in potassium transporter (TRK1, TRK2) -deficient mutants to perform the yeast growth assay. Yeasts expressing ABCG33 and ABCG37 could not grow in the absence of potassium, while yeasts expressing high-affinity potassium transporters could grow (Fig. 5). These results confirm that ABCG33 and ABCG37 are not involved in potassium transport.

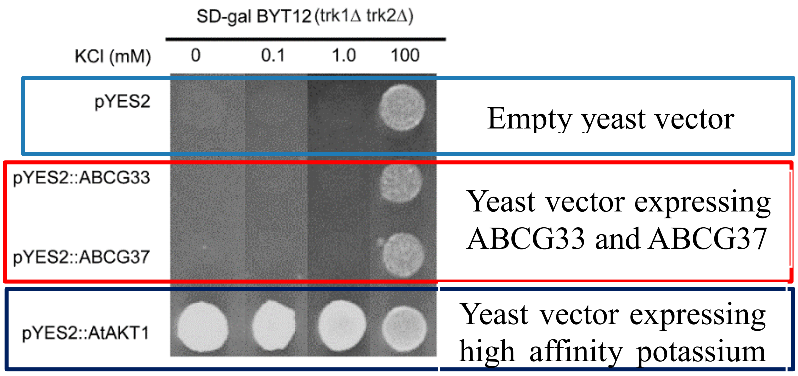

Figure 5. ABCG33 and ABCG37 do not uptake potassium and cannot grow in potassium deficient medium.

In this study, for the first time in the world, we demonstrated the existence of a potassium-independent cesium transport pathway, and identified the potassium-independent cesium uptake proteins ABCG33 and ABCG37. This discovery is believed to contribute significantly to the realization of a complete phytoremediation method for contaminated soil.

3. Conclusion

We succeeded in identifying the world's first potassium-independent cesium uptake protein, paving the way for the realization of a complete phytoremediation method.

4. Future perspective

In this study, we were able to identify potassium-independent cesium uptake proteins in plants. This revealed a new mechanism for absorbing cesium from the soil to the plant body. If ABCG33 and ABCG37 and their homologous genes can be overexpressed in plants, these plants can be effectively used for phytoremediation of radioactive cesium (Fig.6). In future, we would like to focus on the removal of harmful metals, including cesium and continue our research aiming at establishing a new phytoremediation system.

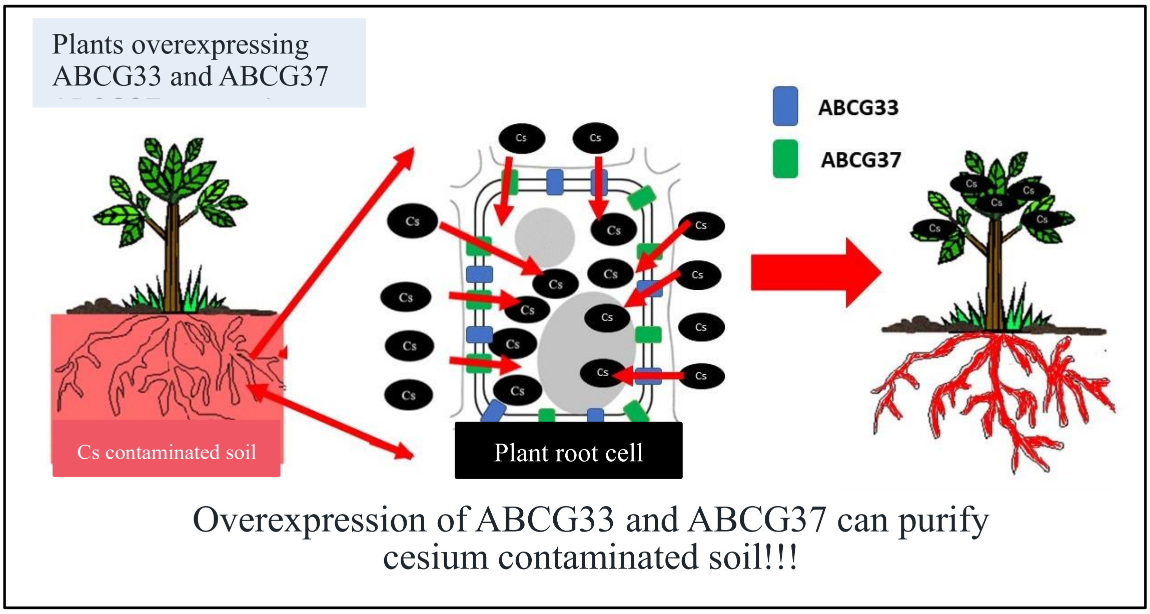

Figure 6. Plants overexpressing ABCG33 and ABCG37 may help in cleaning up the cesium contaminated soil.

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Glossary

Note 1: **Phytoremediation:** Phytoremediation is a new eco-friendly green technique to clean up soil pollutants using plants without significantly changing the soil environment.

Note 2: **ABC protein:** ABC protein is a non-selective transport protein ubiquitously present from bacteria to animals. For example, there are 49 types of ABC proteins in humans and 130 types of ABC proteins in the model plant *Arabidopsis thaliana*.

Note 3: **Screening:** Screening is a method by which the response of plants to various chemicals, stresses is assessed. Using this method, the target genes for the chemicals or stresses can be found.

Note 4: **Arabidopsis:** The scientific name is *Arabidopsis thaliana*. This is used as a model plant for dicotyledon. The plant is small, easy to handle, can be grown anywhere, the life cycle is fast and easy to transform with genetic material. For monocotyledon, rice is used as a model plant.

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