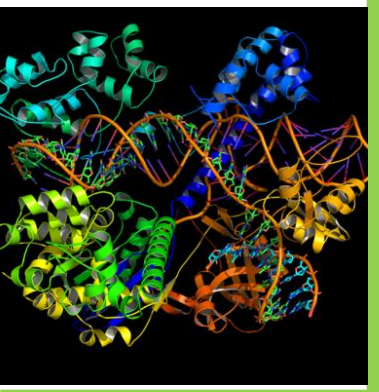


Defining a gene function by mutational studies in plant

Overview of the lecture

- Brief background on molecular biology

  DNA, genetic materials, mechanism of transfer of genetic information

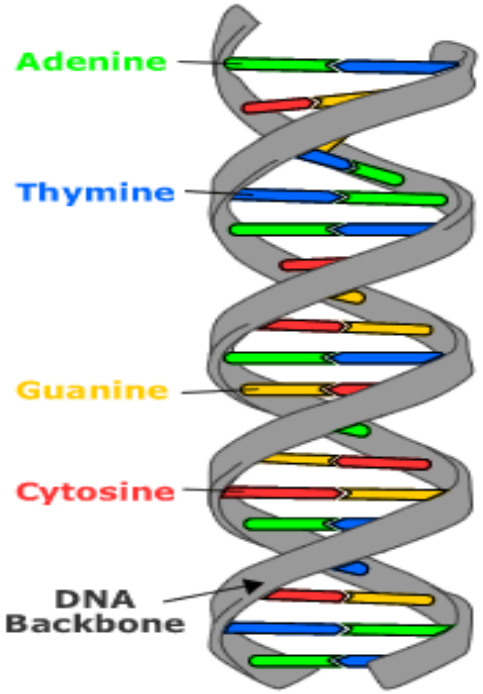
- How to elucidate the gene in the mutant plant

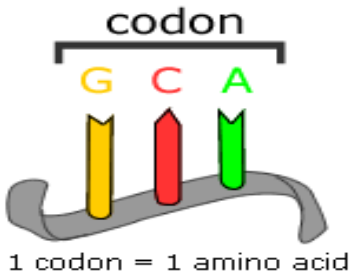
- Making the audience aware about the importance and power of molecular biology in enhancing the quality of our life

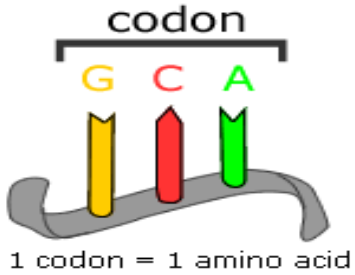
Iwate University 岩手大学

- Plants are built with the same genetic material that we are made of
- Like human or animals, they have chromosomes, where all the genetic information are stored
- To process the genetic information the plants and animals essentially use the same mechanism
- Finally, like human or animal the plant also respond to different stimului such as light, temperature, gravity etc.

Iwate University 岩手大学







Deoxyribonucleic acid, or **DNA**, is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms

Iwate University 岩手大学

More information on DNA

The main role of DNA molecules is the long-term storage of information

DNA contains the instructions needed to construct other components of cells, such as proteins and RNA molecules. It is often called the blue print of life

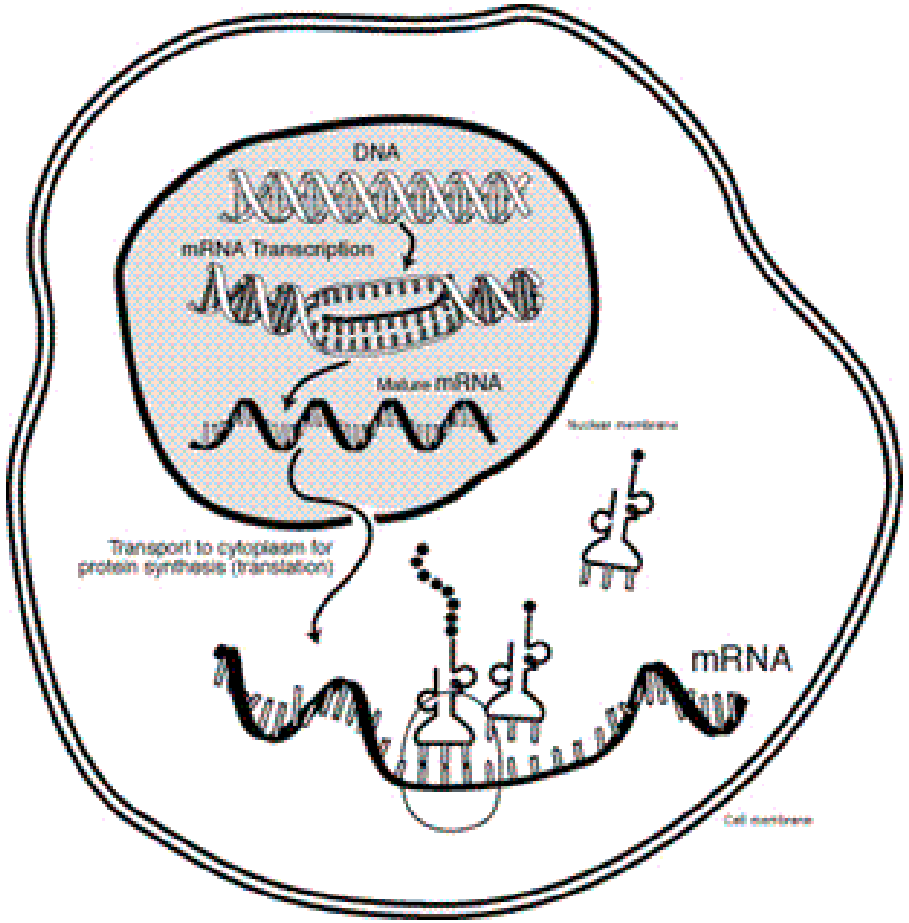
The DNA segments that carry this genetic information are called genes

The other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information

Within cells, DNA is organized into structures called chromosomes and the set of chromosomes within a cell make up a genome

Arabidopsis chromosome

Mechanism of transfer of genetic information



DNA

↓ transcription

RNA

↓ translation

Protein

Iwate University 岩手大学

DNA

Genes

Chromosome

Genome

Mechanism of transfer of

genetic material

Plants response to different environmental stimului

Arabidopsis germination

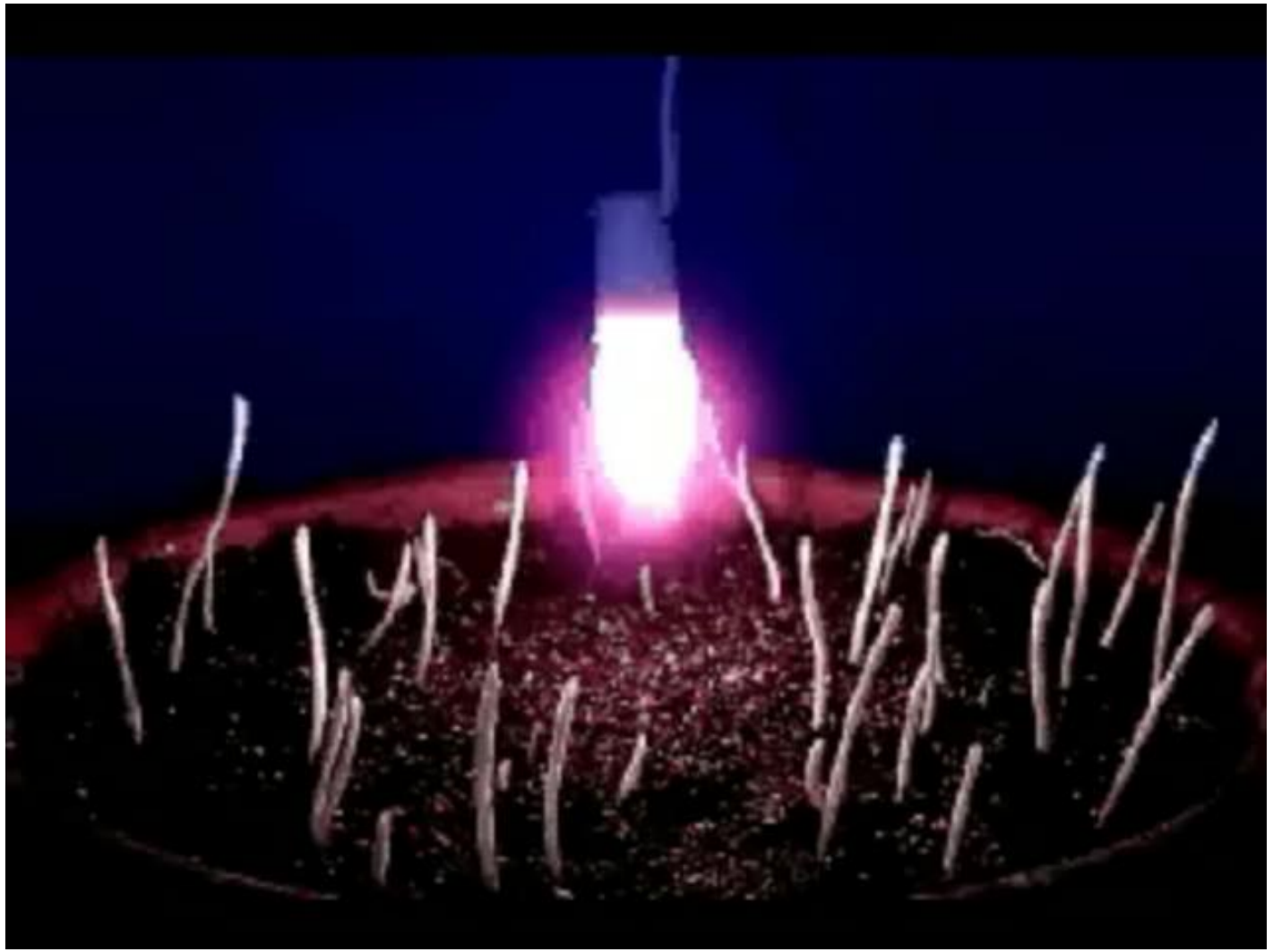
Courtesy: Roger Hangarter

Germination of Sunflower in light and dark

Courtesy: *Roger Hangarter*

Corn phototropism

Courtesy: Roger Hangarter



Response to light

Gravity response in root and shoot

Courtesy: *Roger Hangarter*

Sunflower
solar track

Iwate University
岩手大学

Questions:

1) What determines such responses of plants?

2) Is it possible to find out the factors that influence the plant responses to different stimulus?

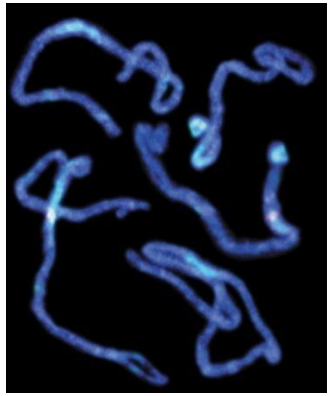
Iwate University 岩手大学

Model Plant

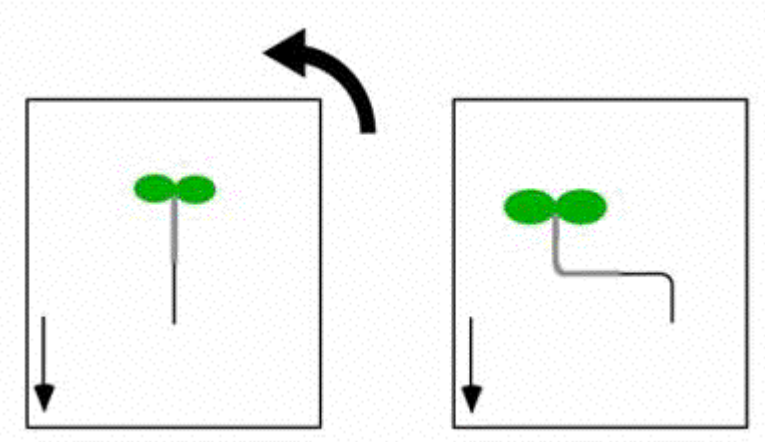
Arabidopsis thaliana

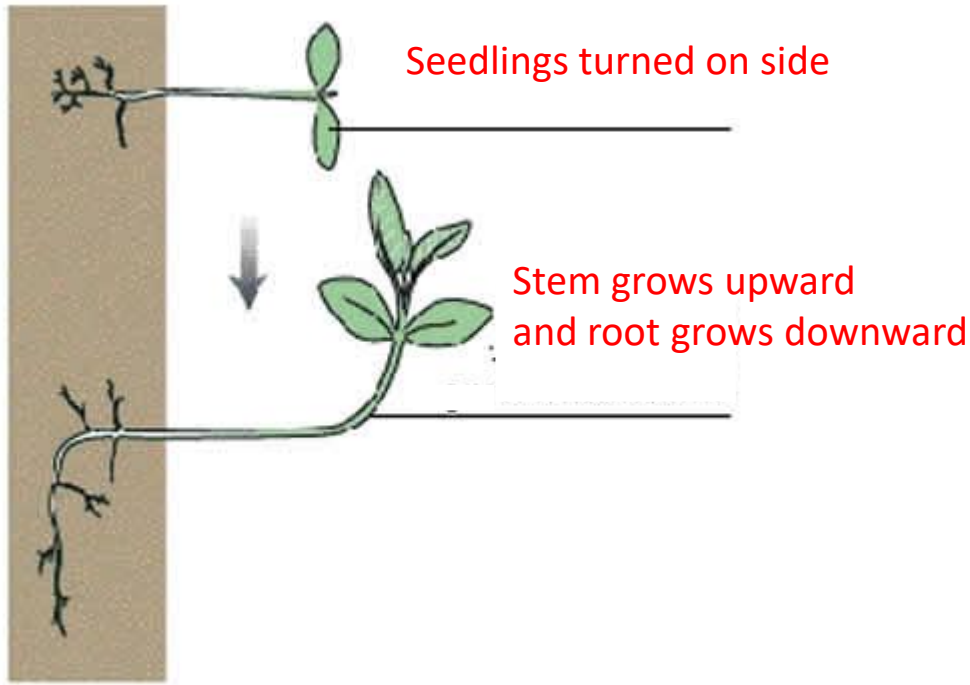
- ★ *Arabidopsis thaliana* has a small genome size
- ★ The whole genome has been sequenced
- ★ The genome of this plant is composed of 5 chromosomes which contains 125 Mb of DNA and 25,948 identified proteins
- ★ It has a shorter life cycle relative to other plants; Takes only six weeks from germination to produce seeds
- ★ It is easy to grow. Virtually it grows everywhere
- ★ Finally transferring the gene in this plant is relatively easy compared to other plants

Arabidopsis chromosomes



Iwate University 岩手大学

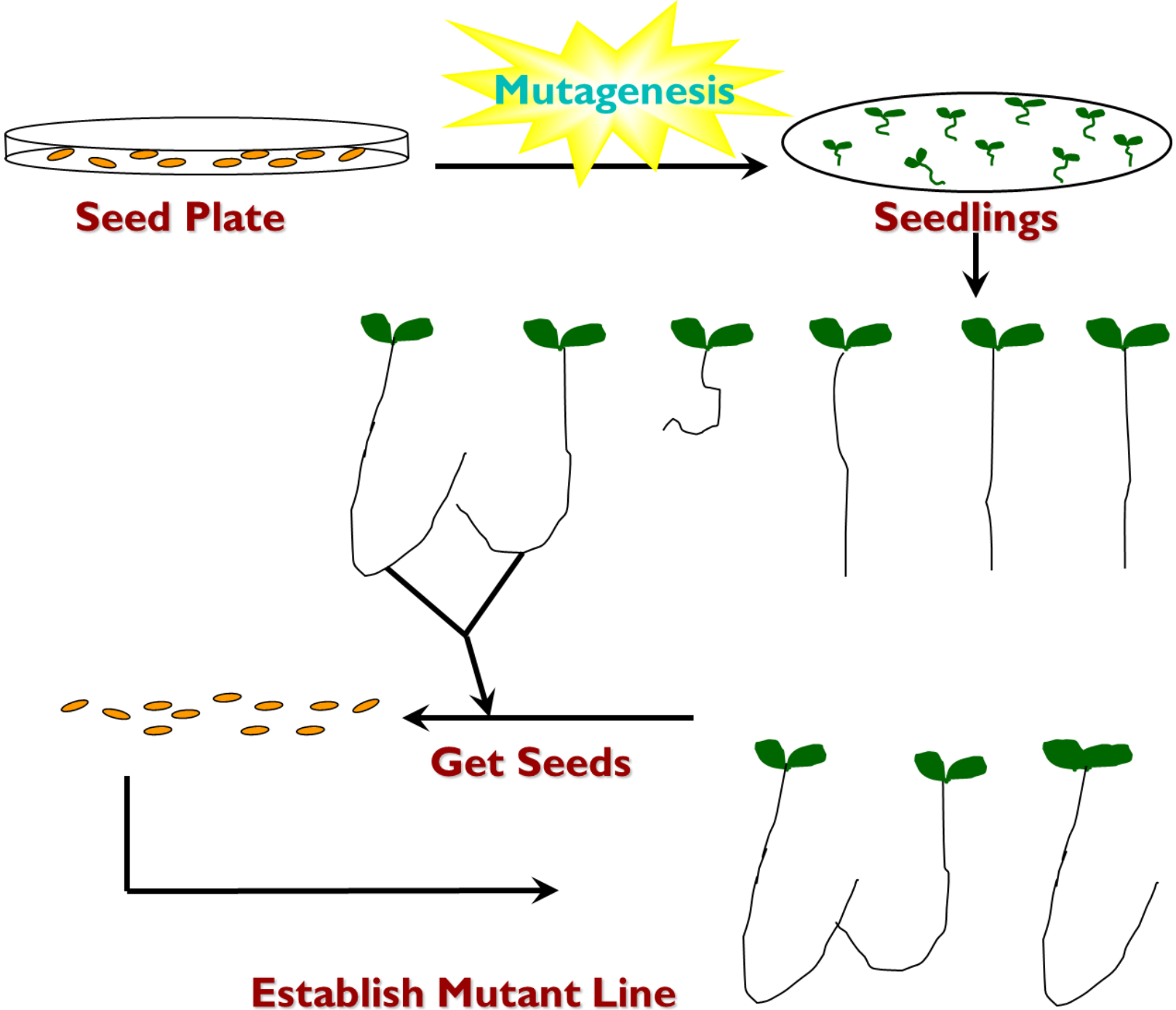




Seedlings turned on side

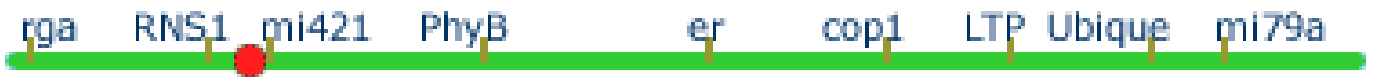
Stem grows upward
and root grows downward

Iwate University
岩手大学


Mutagenesis
Seed Plate
Seedlings
Get Seeds
Establish Mutant Line

Chromosome

1



2

3

4

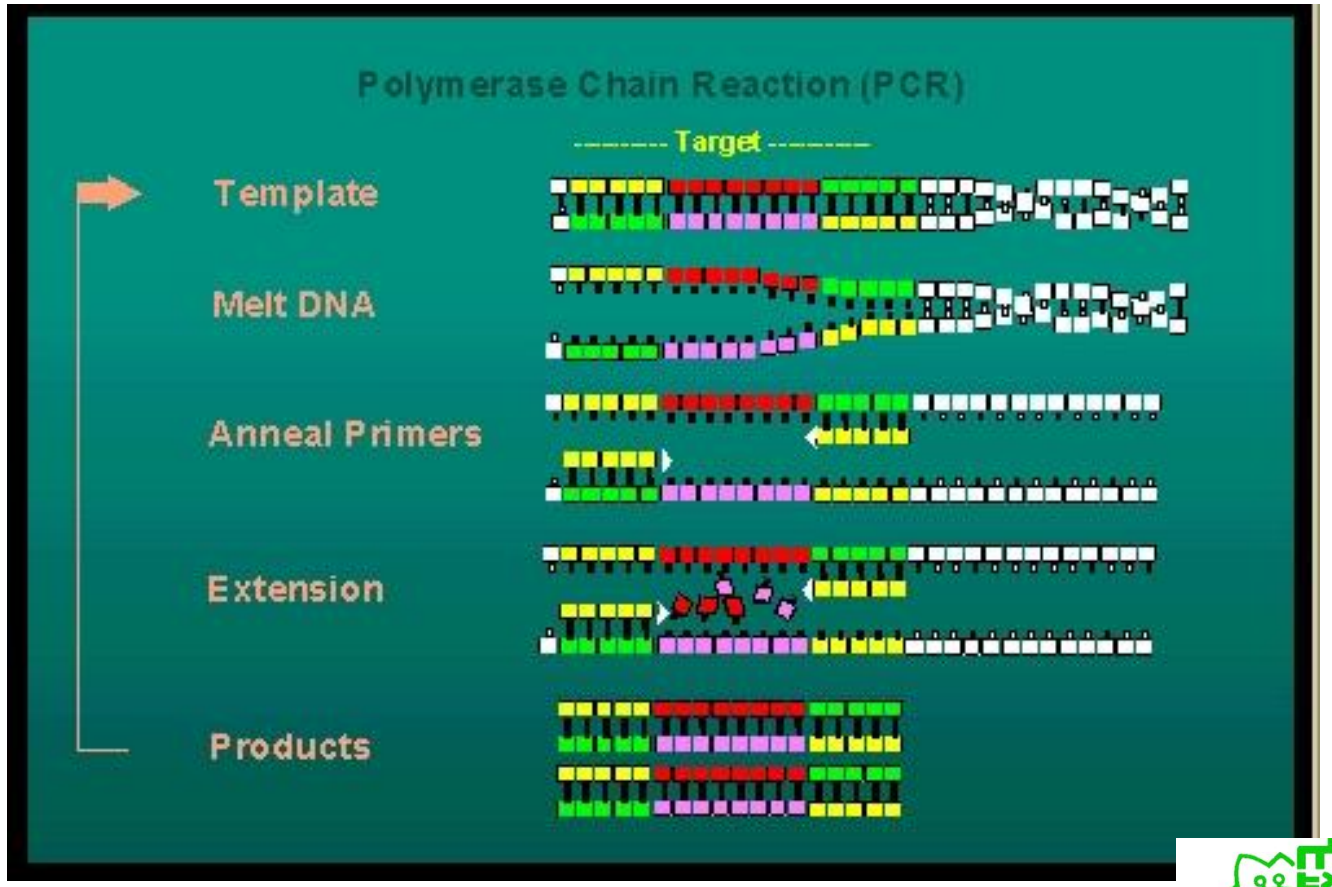
5

Iwate University 岩手大学

Polymerase chain reaction (PCR) based mapping

- Polymerase chain reaction (PCR) is a molecular biology technique and used to amplify specific regions of a DNA strand. This can be a single gene, just a part of a gene, or a non-coding sequence.

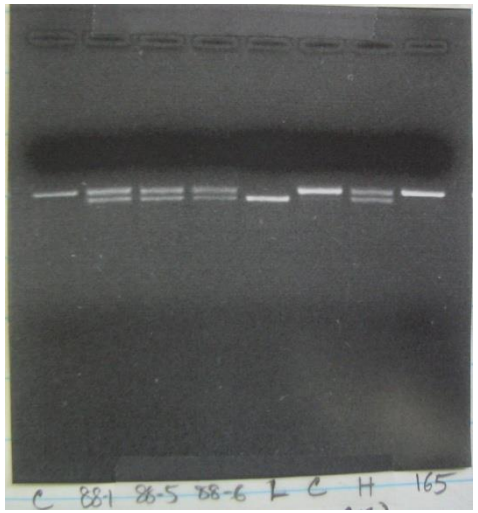
- It was Invented in 1983 by Dr. Kary Mullis. He later got Nobel prize for this invention



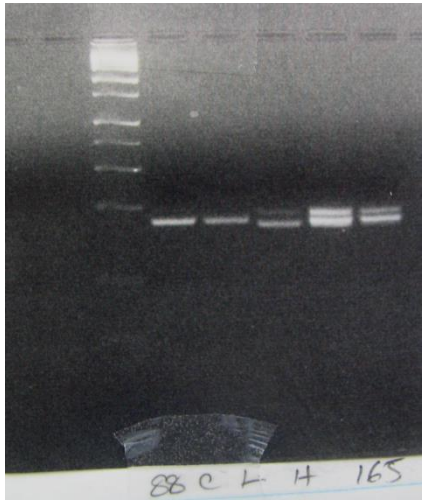
Iwate University 岩手大学

https://www.youtube.com/watch?v=iQsu3Kz9NYo

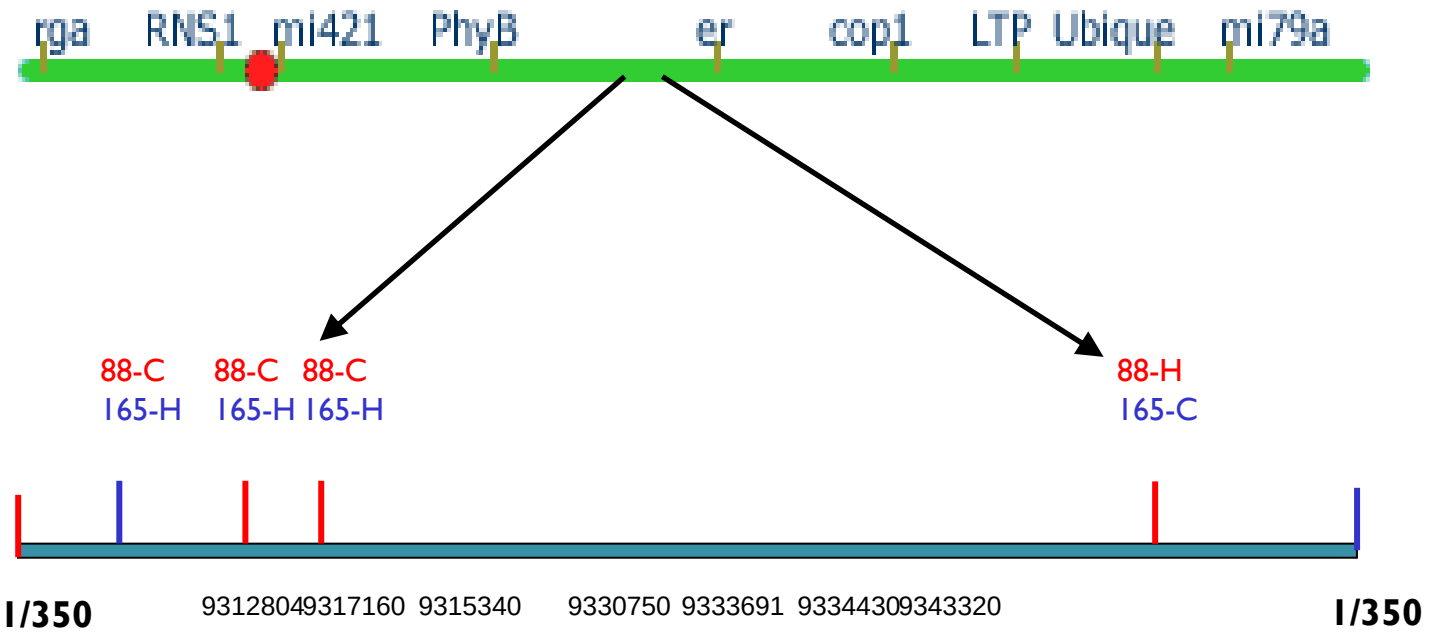
Iwate University 岩手大学



F7D8-1: 9354750 Mb



F7D8-7: 9321804 Mb



1) Identify the mutation by sequencing

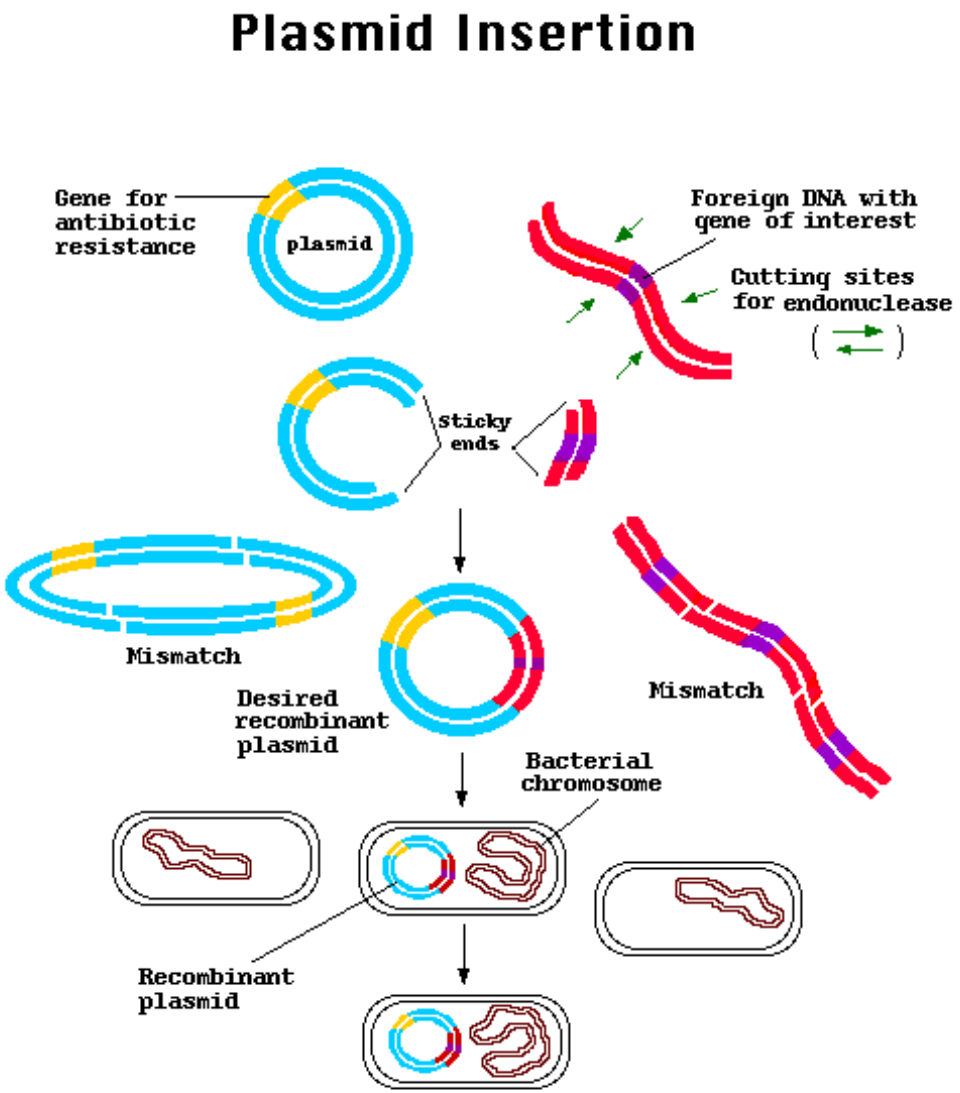
EIR1- chromosome 5 Marker- CTR1 and CIW14

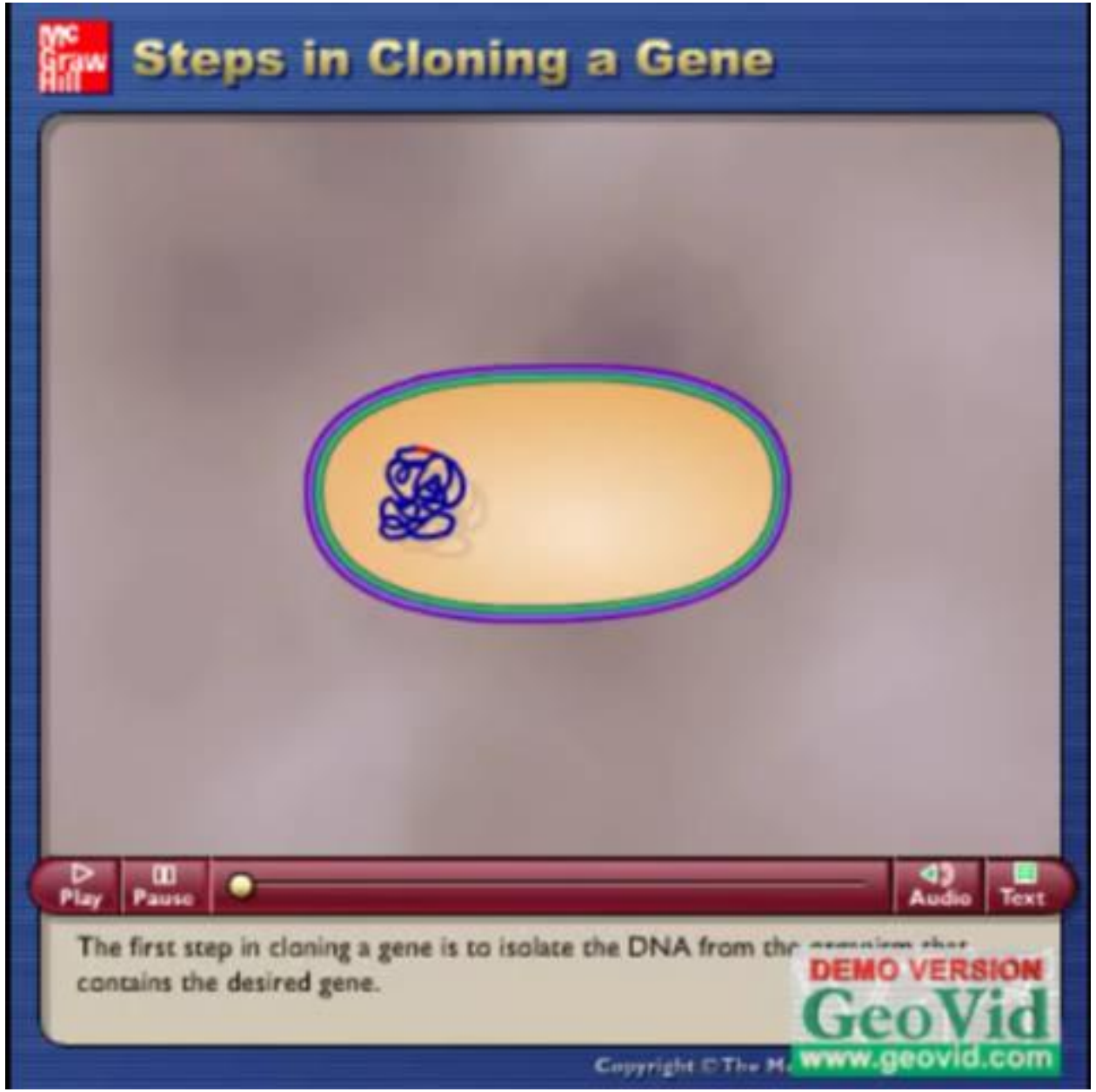
Gene ID- At5g57090

2) Complementation test

Inserting the wild-type gene in the mutant to see whether it can restore the abnormal phenotype

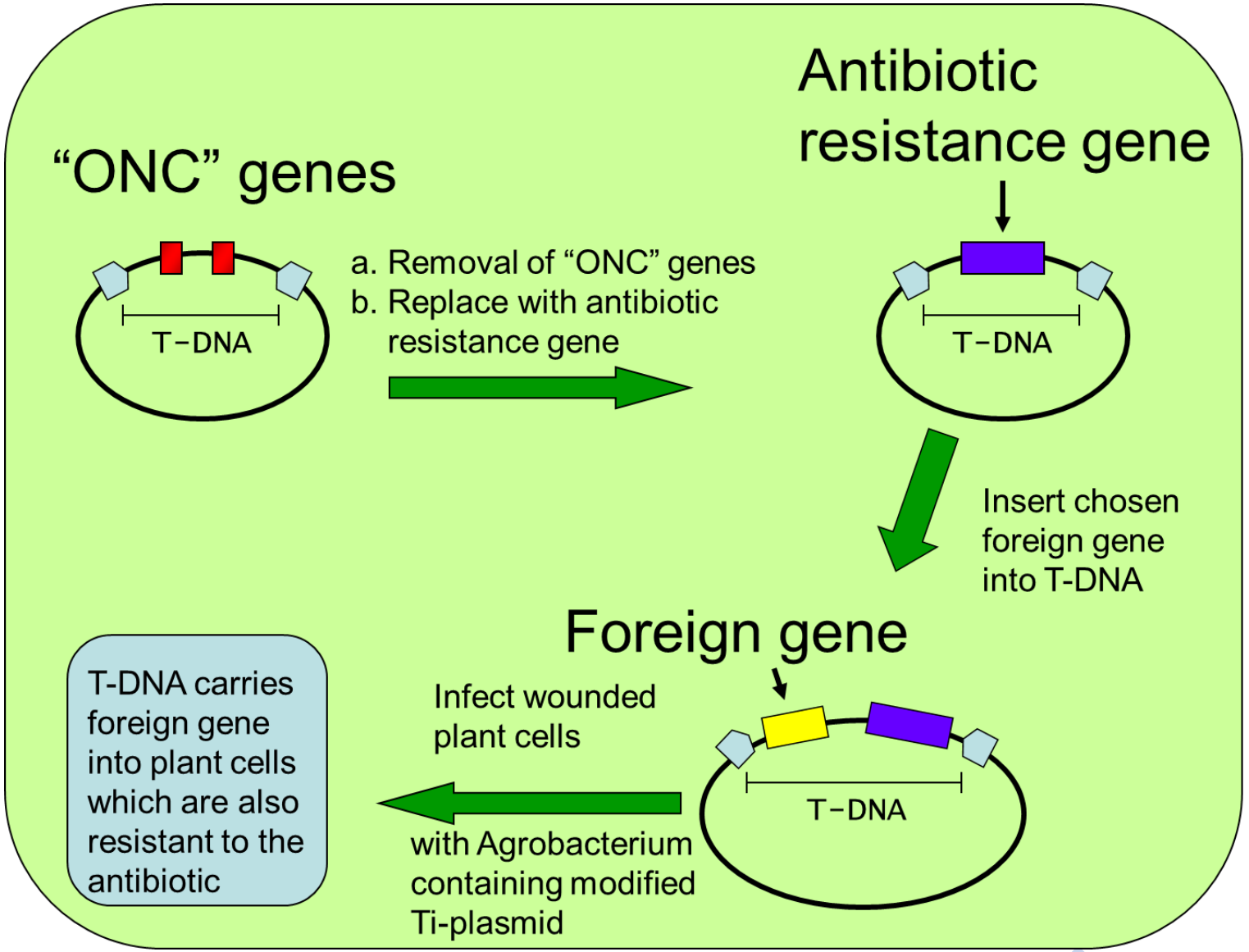
Steps in gene cloning

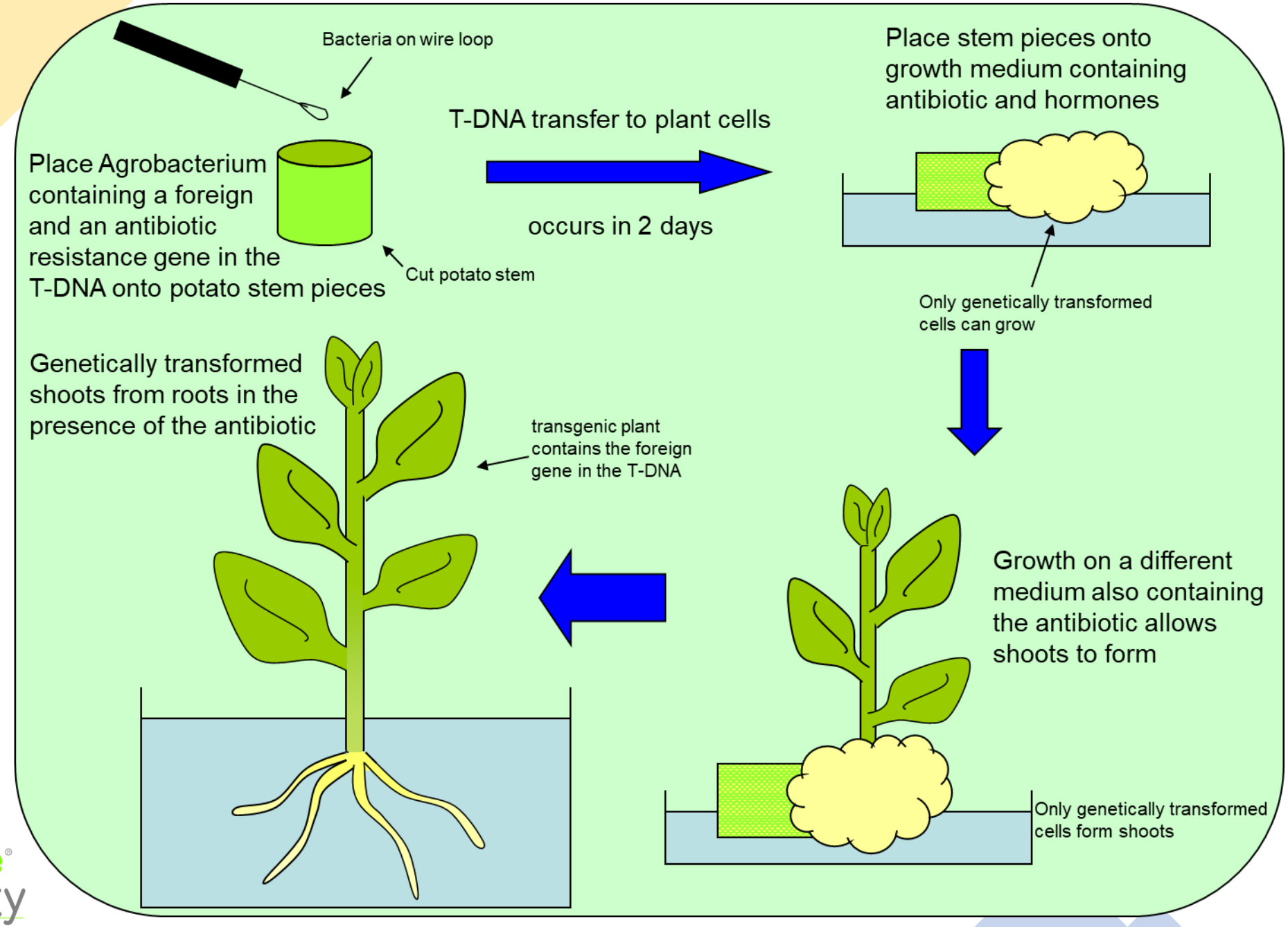




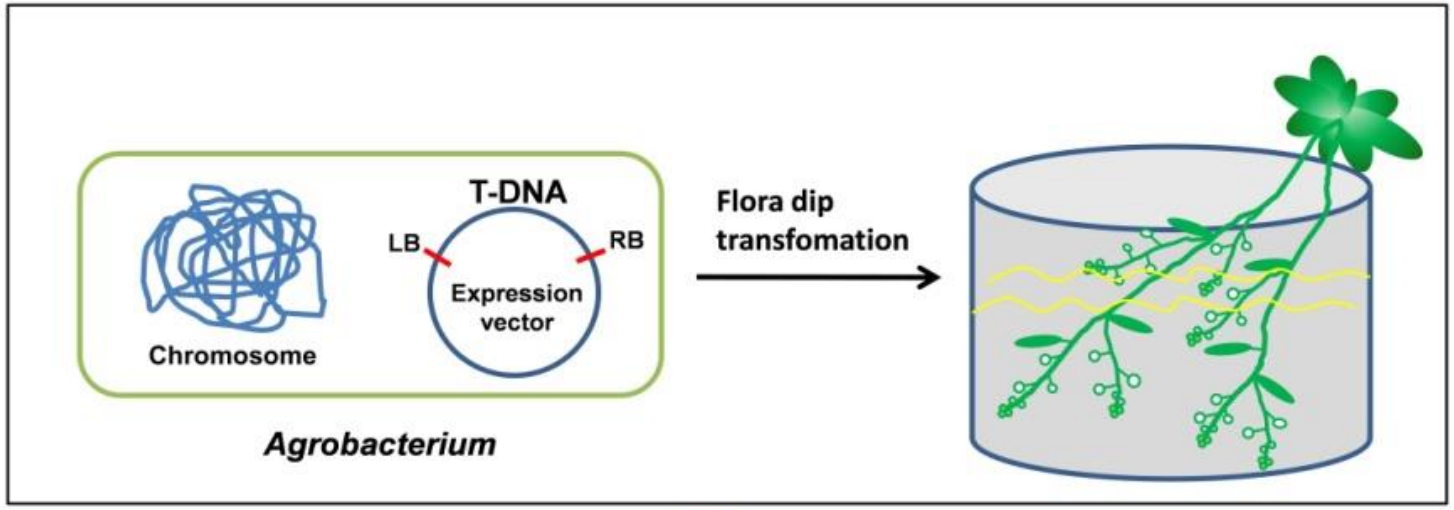
Once the gene is cloned in a vector, next step is to transfer the gene to the mutated plants

For transferring the gene, we use a natural soil bacteria, Agrobacterium and the process is called agrobacterium mediated transformation


"ONC" genes
T−DNA
a. Removal of "ONC" genes
b. Replace with antibiotic resistance gene
Antibiotic resistance gene
T−DNA
Insert chosen foreign gene into T-DNA
Foreign gene
T−DNA
Infect wounded plant cells
with Agrobacterium containing modified Ti-plasmid
T-DNA carries foreign gene into plant cells which are also resistant to the antibiotic


Bacteria on wire loop
Place Agrobacterium containing a foreign and an antibiotic resistance gene in the T-DNA onto potato stem pieces
Cut potato stem
T-DNA transfer to plant cells
occurs in 2 days
Place stem pieces onto growth medium containing antibiotic and hormones
Only genetically transformed cells can grow
Growth on a different medium also containing the antibiotic allows shoots to form
Only genetically transformed cells form shoots
transgenic plant contains the foreign gene in the T-DNA
Genetically transformed shoots from roots in the presence of the antibiotic

Floral dip Transformation



Screen with antibiotics

Grow plants to collect seeds

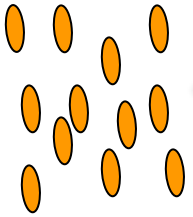
Final step: to validate the gene function, we need to restore the mutant phenotype

Transfer of EIR1 gene

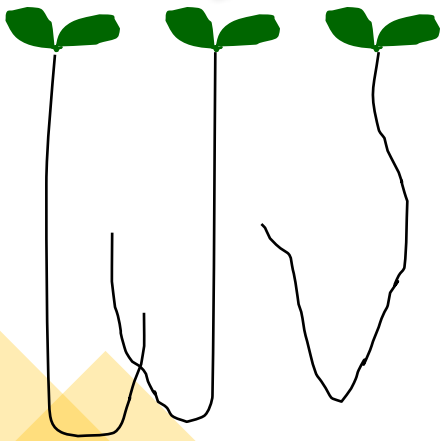
to mutant plants by Agrobacterium mediated transformation

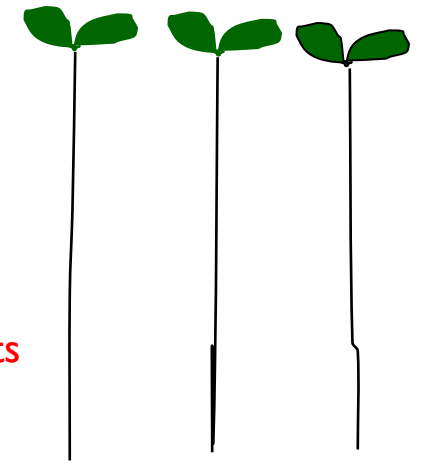
eir1 mutant

Collect seeds



Grow plants to look at the phenotype





Complemented plants

Real life application of gene transfer technique

By using this technique we can speed up our conventional breeding system

Enhance the nutritional value of the crops

Make the crops resistant to different adverse environmental conditions such as cold, drought, salinity, flood etc.

Bio-engineer the plants to detoxify the heavy metal contaminations such as cadmium, mercury, arsenic from soil

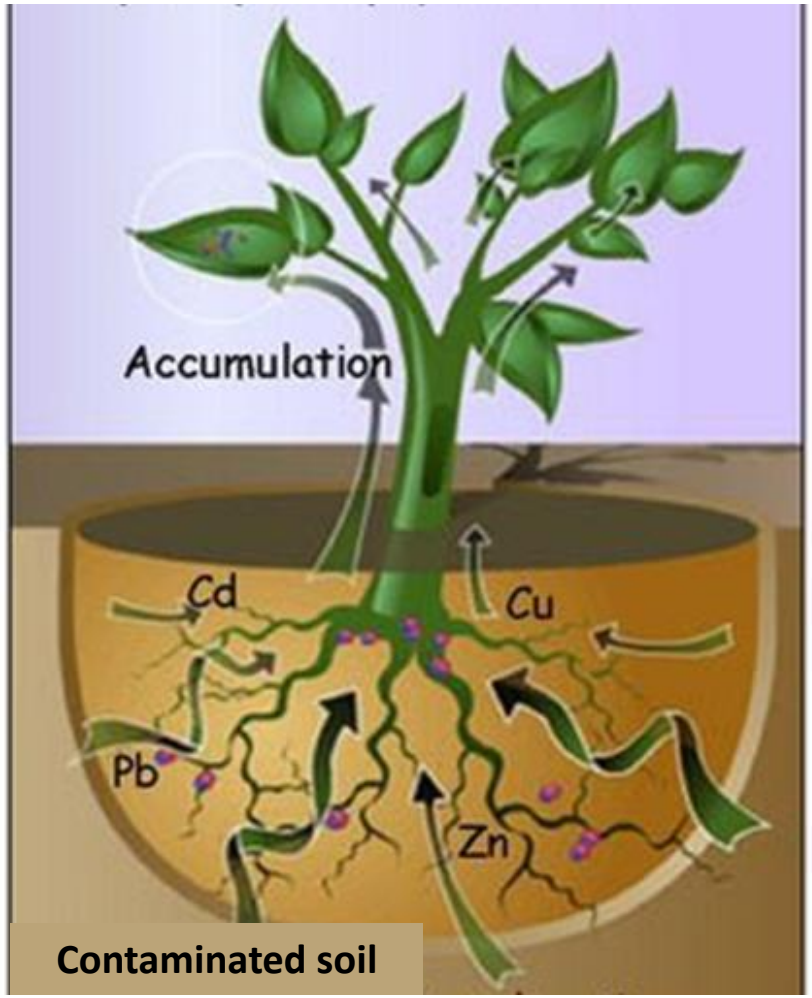
Create the exotic flowers with different colors and fragrance

Produce vaccines in plant leaves

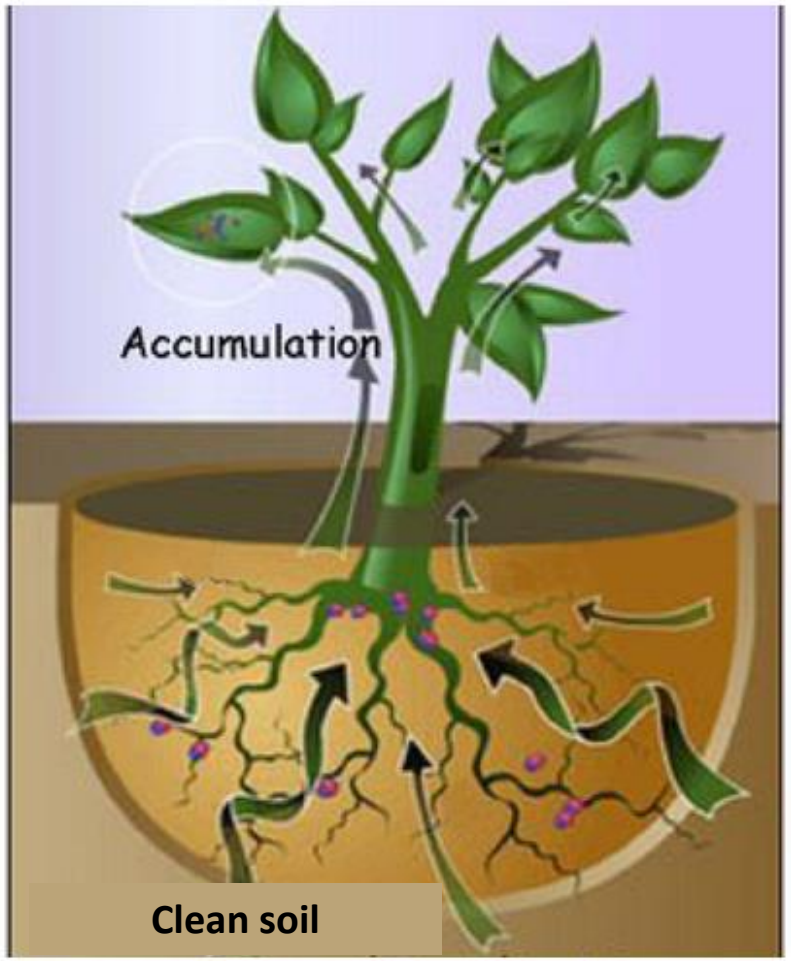
Iwate University 岩手大学

Concept of phytoremediation

Natural plant

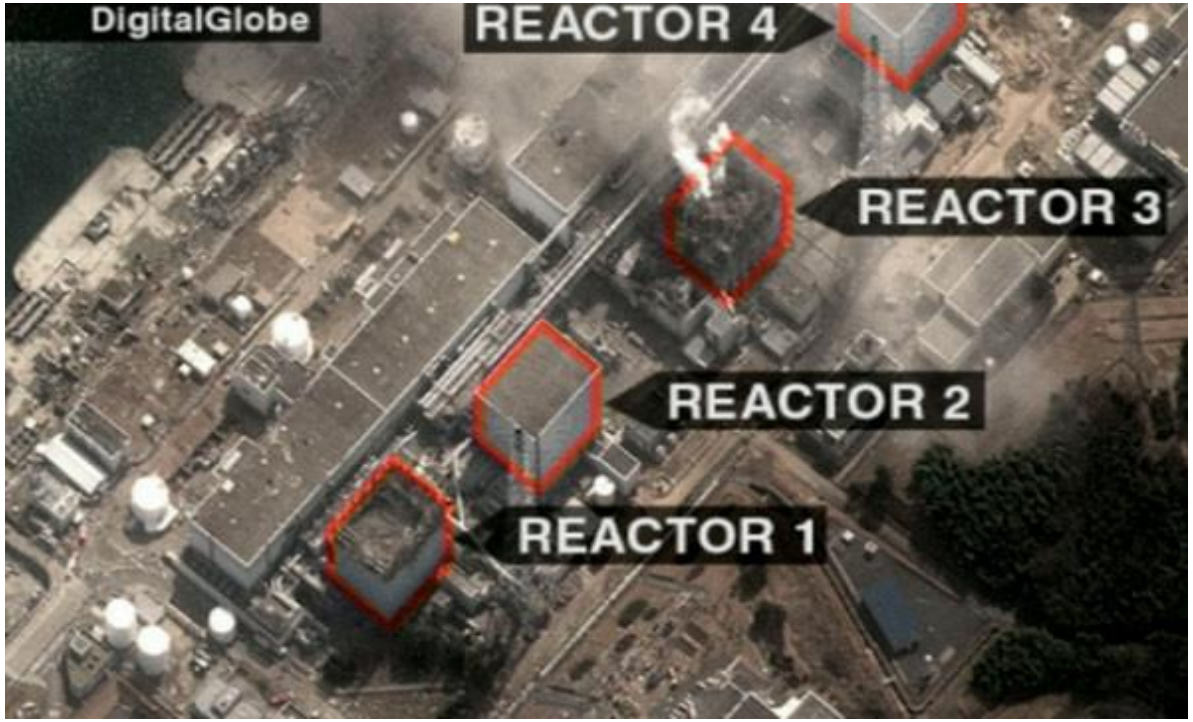


Plants capable of efficiently transporting metals from soil



Iwate University 岩手大学

Chernobyl disaster (1986)



Fukushima disaster (2011)

Isotopes of Cesium

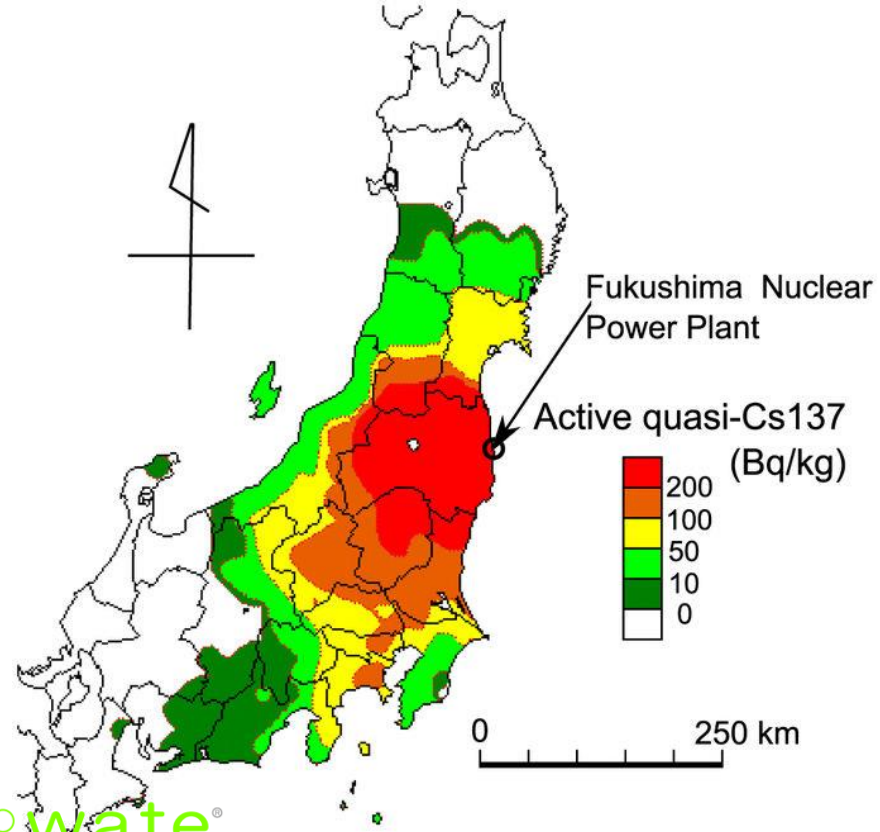
^{133}Cs → Stable isotope

^{134}Cs → 2.0648 years half life

^{137}Cs → 30.17 years half life

Iwate University 岩手大学

Radio cesium spreads from the nuclear plant area and finds its way towards river and agricultural land



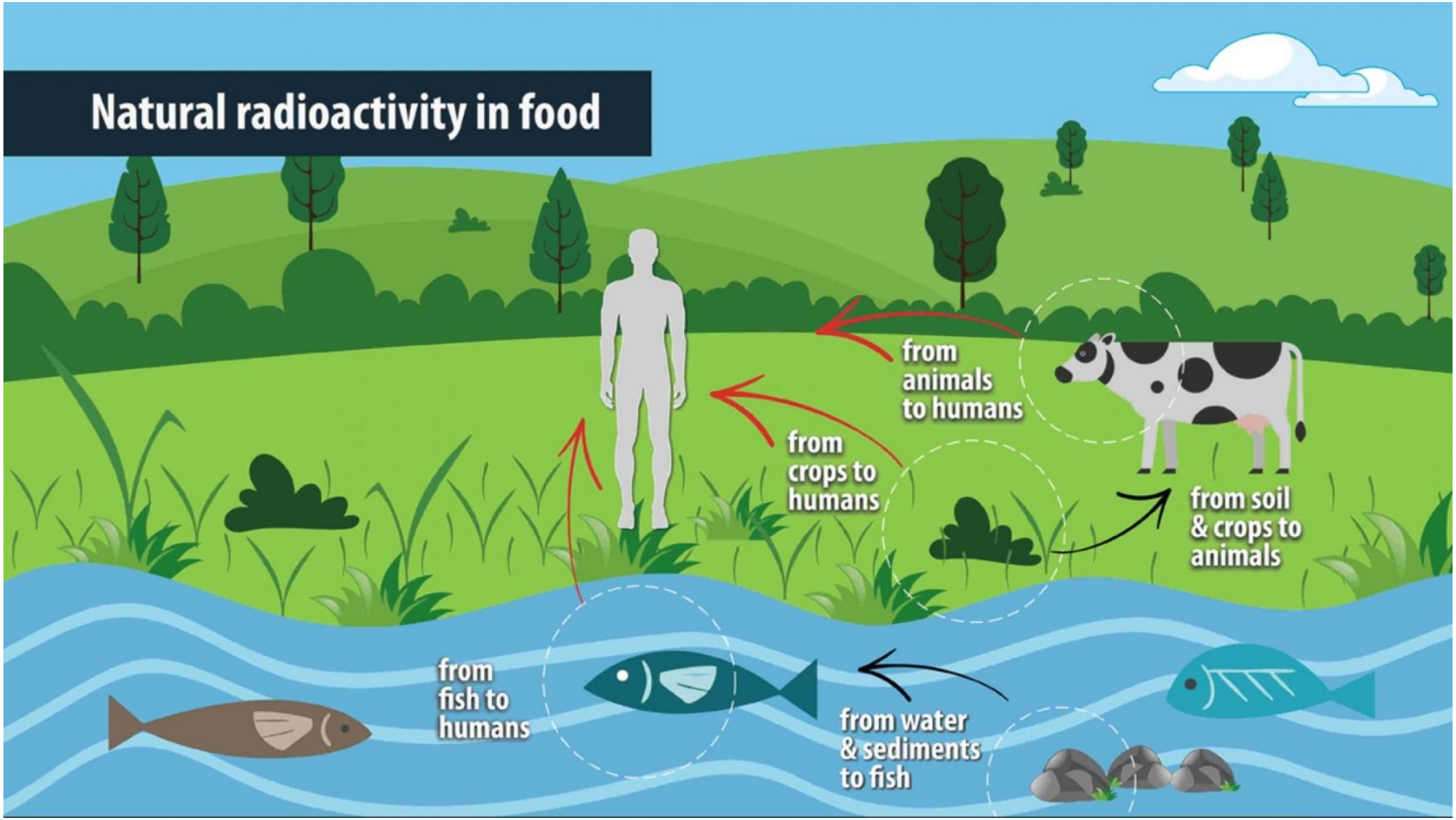
Chernobyl: Cs-137 contamination

1090 square mile "exclusion zone"
>104 Curies/sq mile

3840 square miles strict radiation dose-control zone

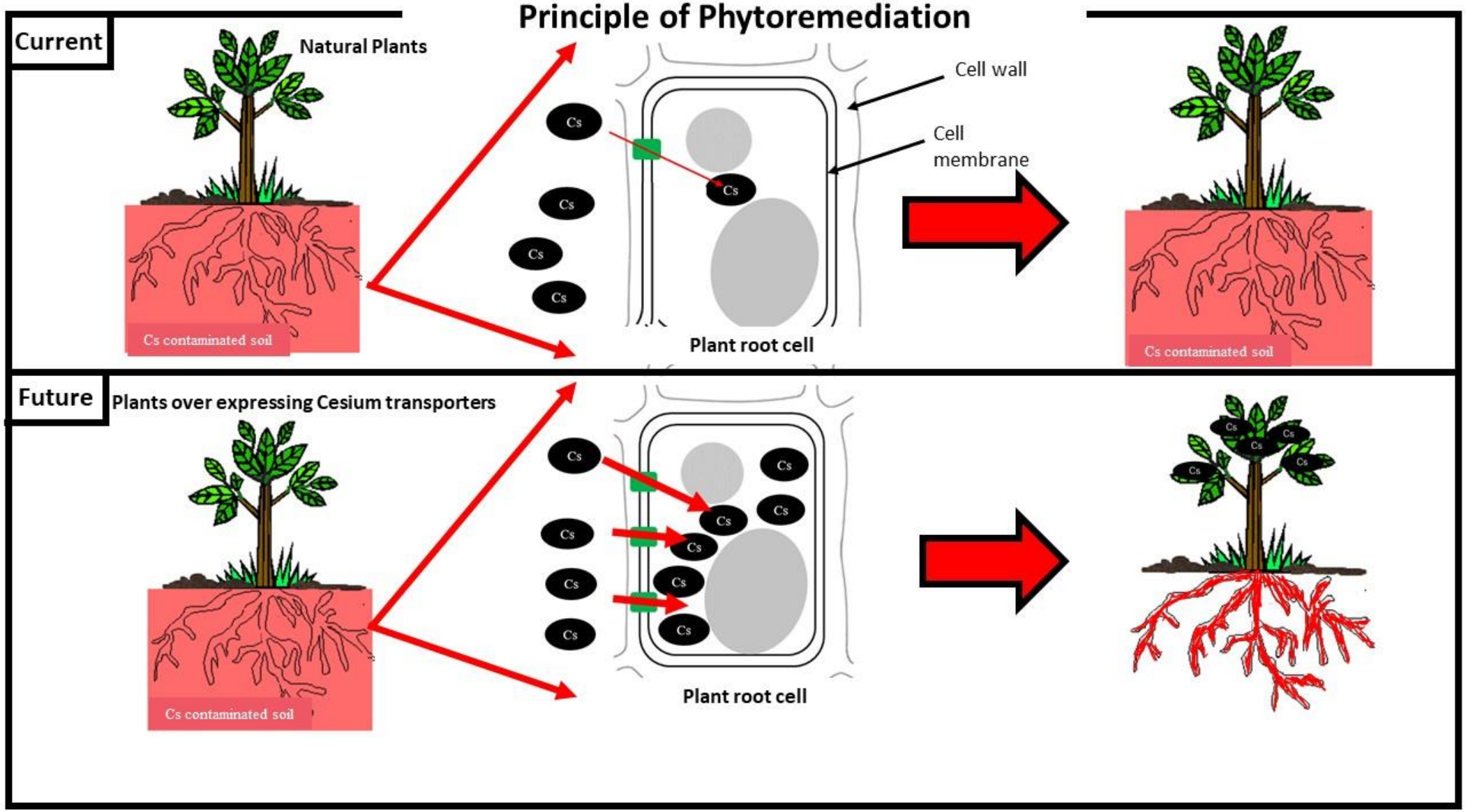
39-104 Curies/sq mile

Iwate University 岩手大学

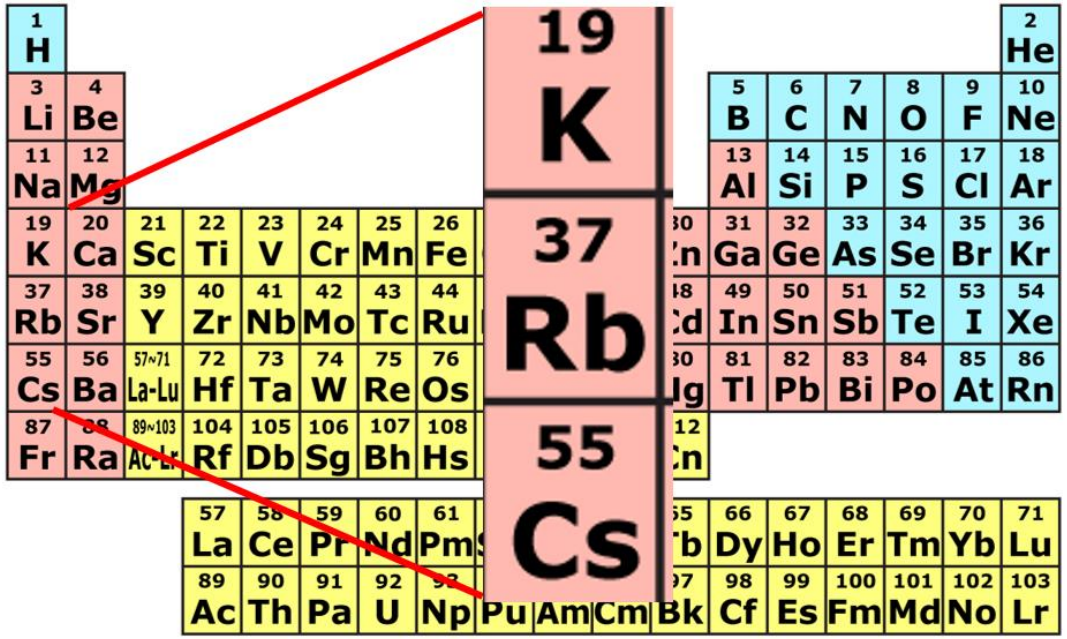


Radio cesium penetrates into our food chain

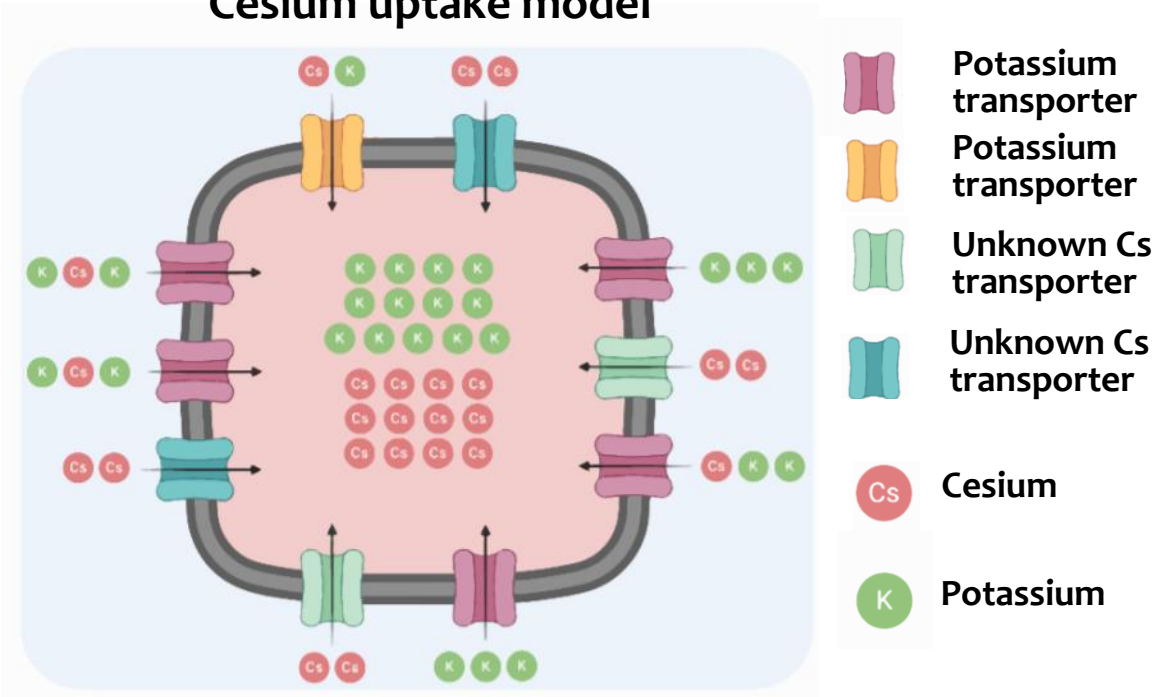
Iwate University 岩手大学



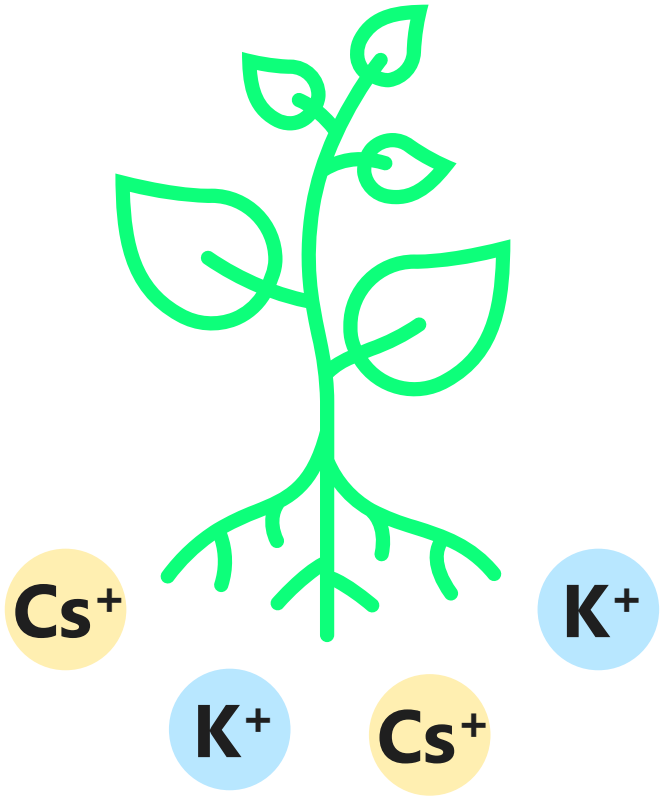
Can we clean up the radiocesium contaminated soil using plant ?



Cesium uptake model

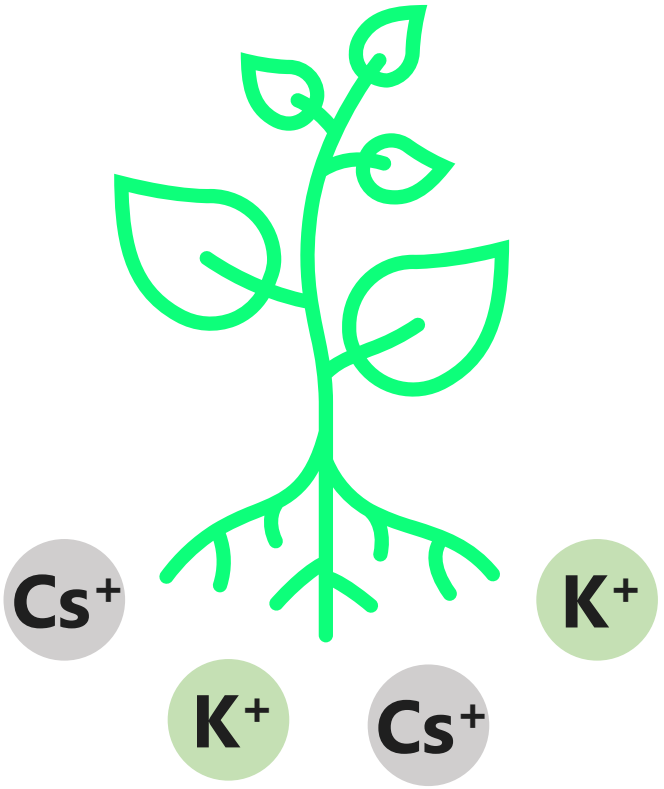


Cs^+ taken up by K^+ Transporters



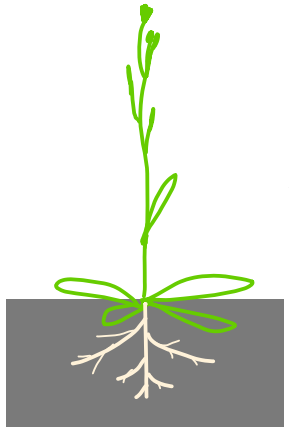
K^+-depleted soil

K^+-independent

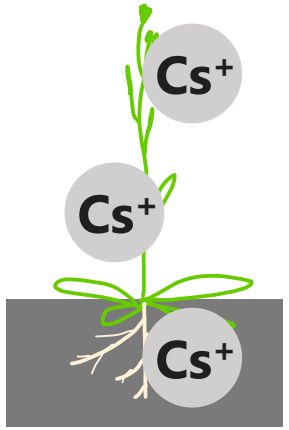


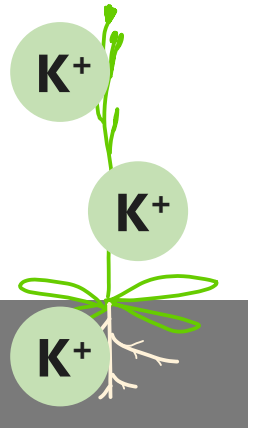
$K^+$is retained in the soil

Are there K^+-independent Cs^+ transporters?

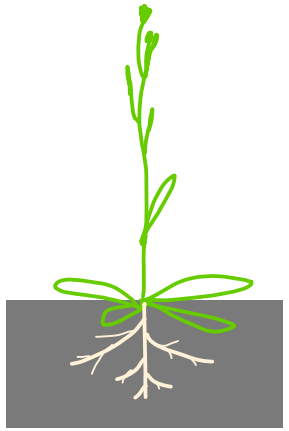


Several **ABC transporters** are differentially expressed

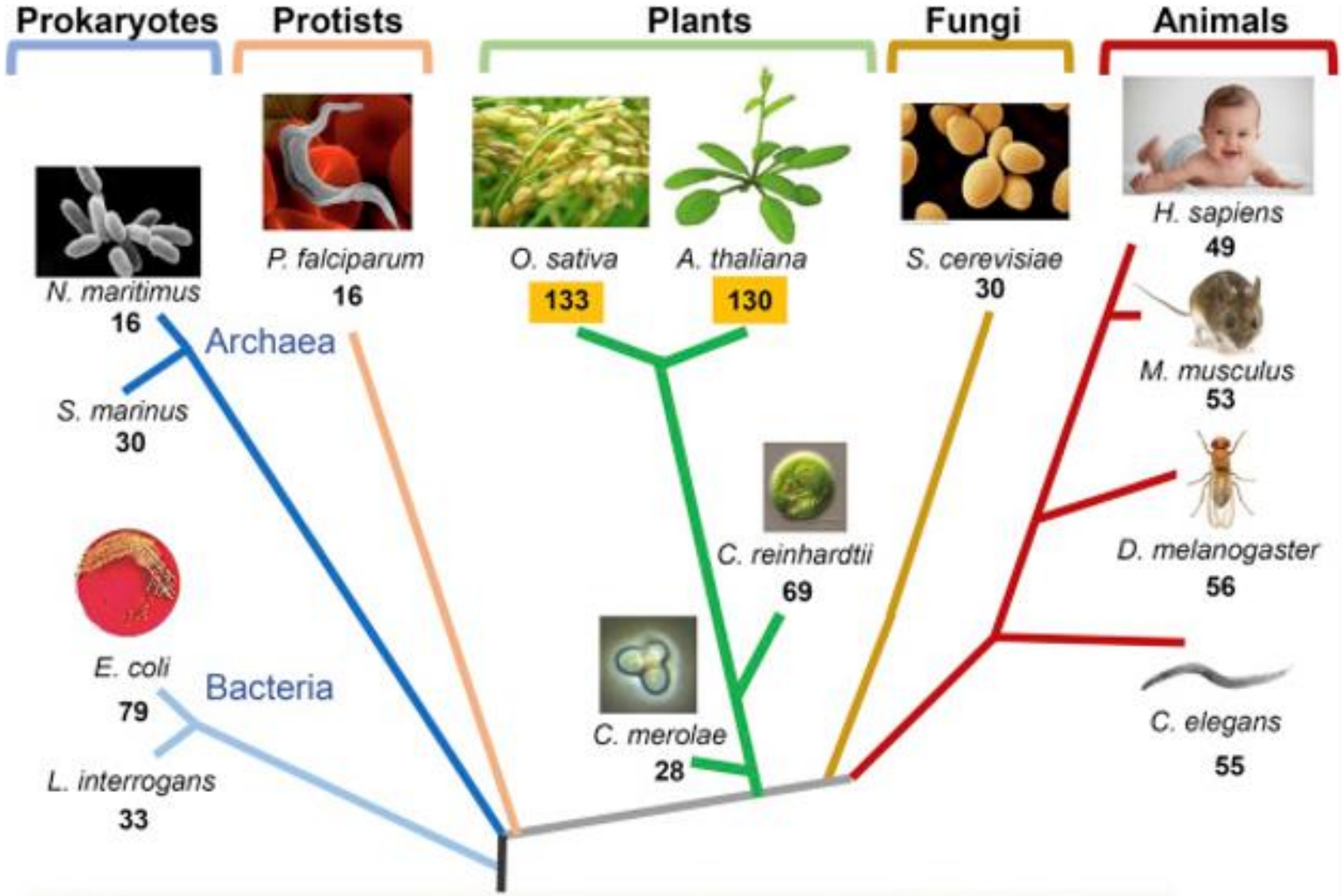




The expression of these ABC transporters are not altered under K^+



Hampton et al., Plant Physiology (2004) Microarray data



Reviewed Hwang et al., Molecular Plant (2016)

ABC transporters

ABCA	12
ABCB	30
ABCC	17
ABCD	2
ABCE	3
ABCF	5
ABCG	43
ABCI	16
Total	130

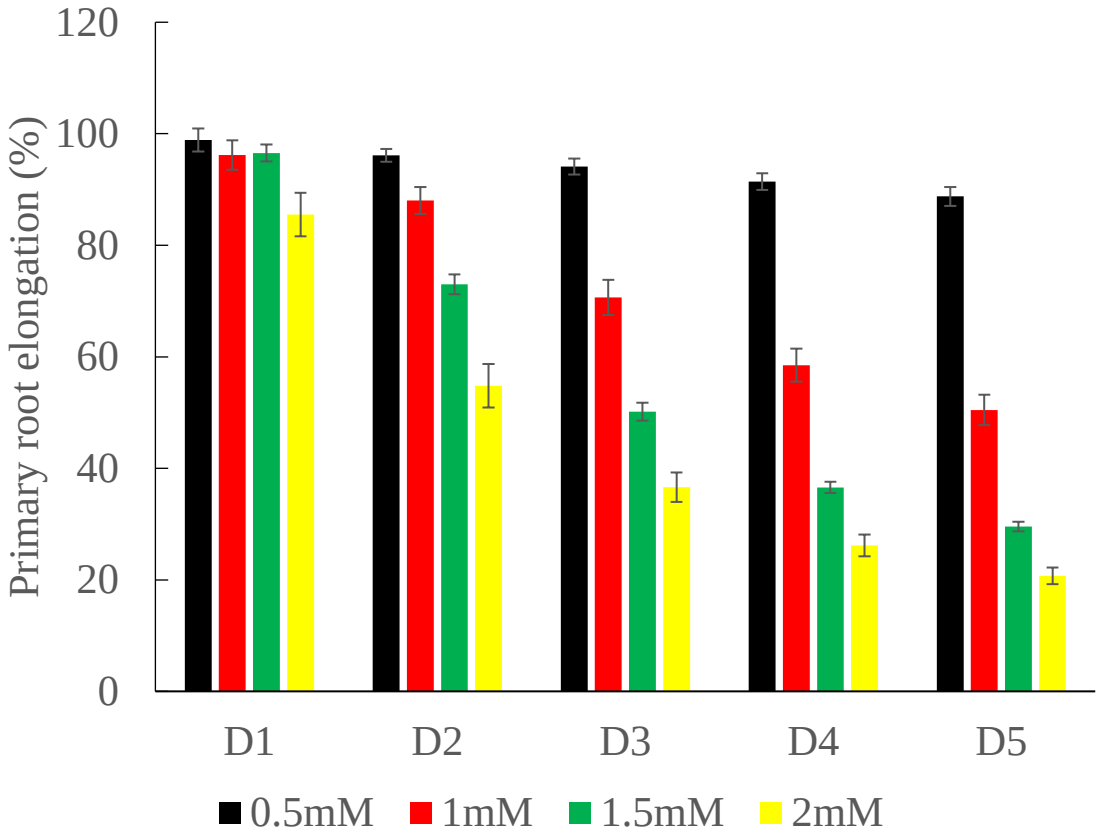
◆ 8 subfamilies

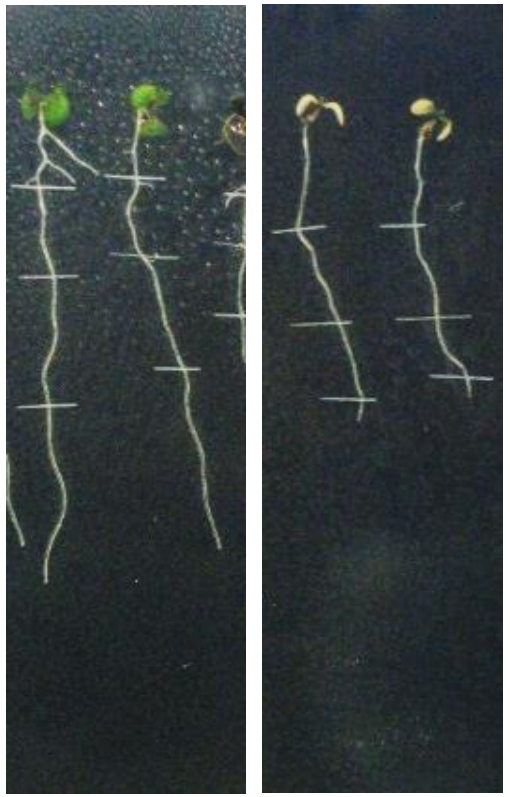
◆ Subfamilies **B**, **C**, and **G**
→ Metal transport activity

◀ Numbers of each subfamily of ABC transporters in *Arabidopsis thaliana*

Non-specific substrate selectivity of ABC transporters

Developing a root screening method

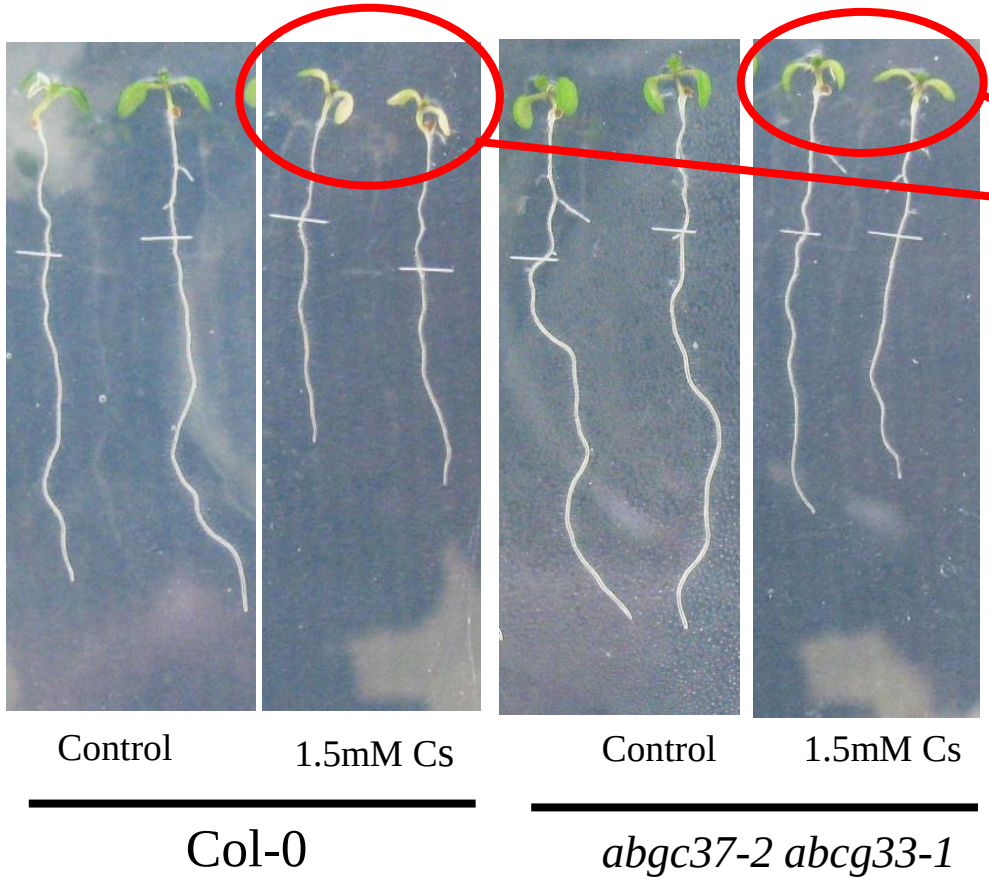




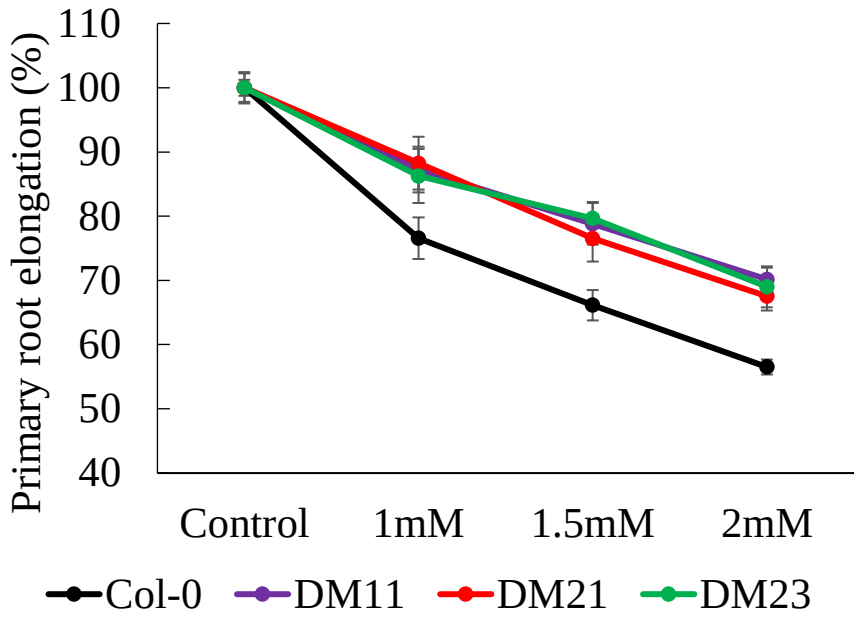
Control Cs

Iwate University 岩手大学

ABCG33 and ABCG37 possibly function as Cs^+ transporters



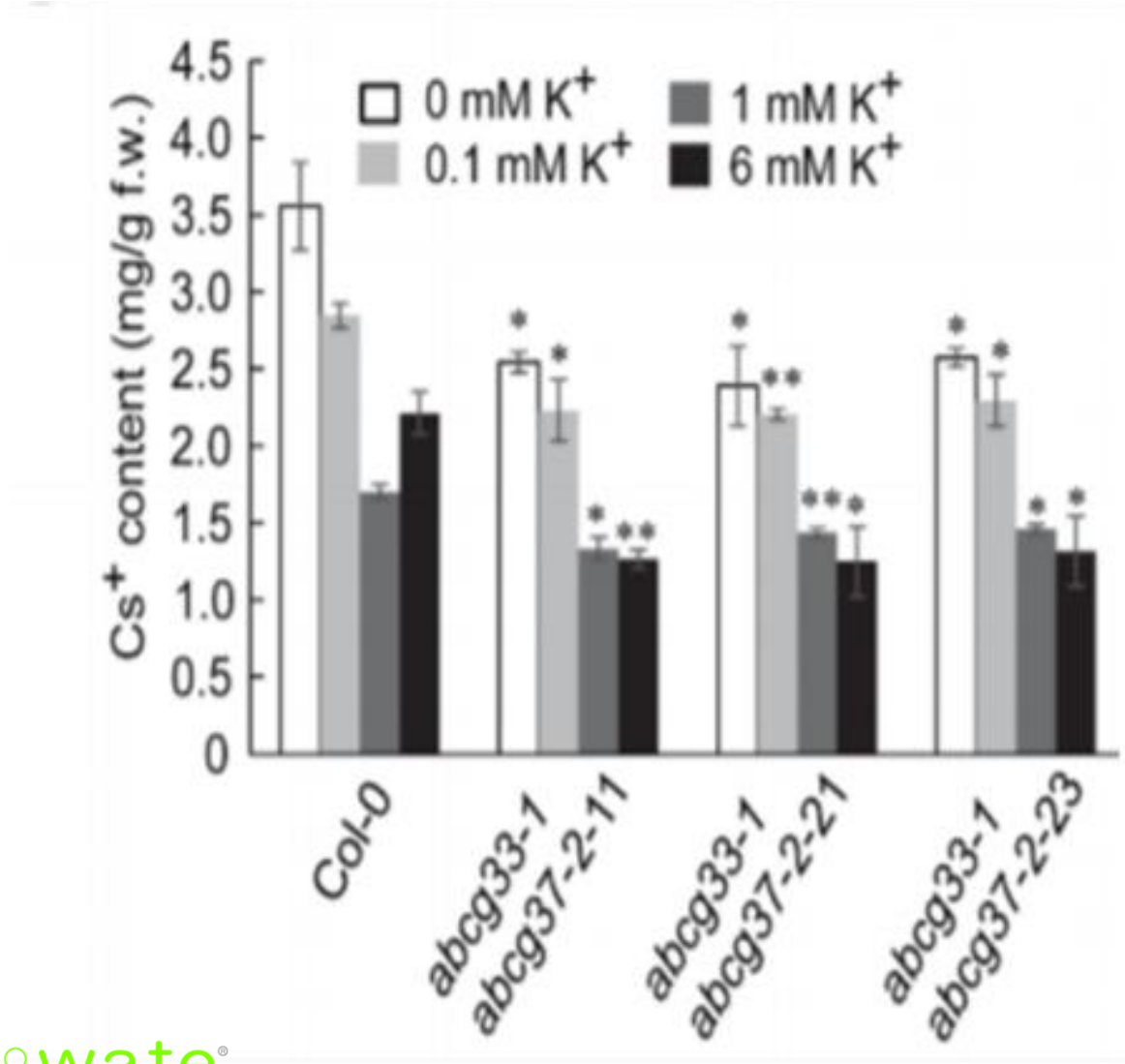
In presence of cesium, the leaves of the double mutant did not turn white and the plant did not die !!!!

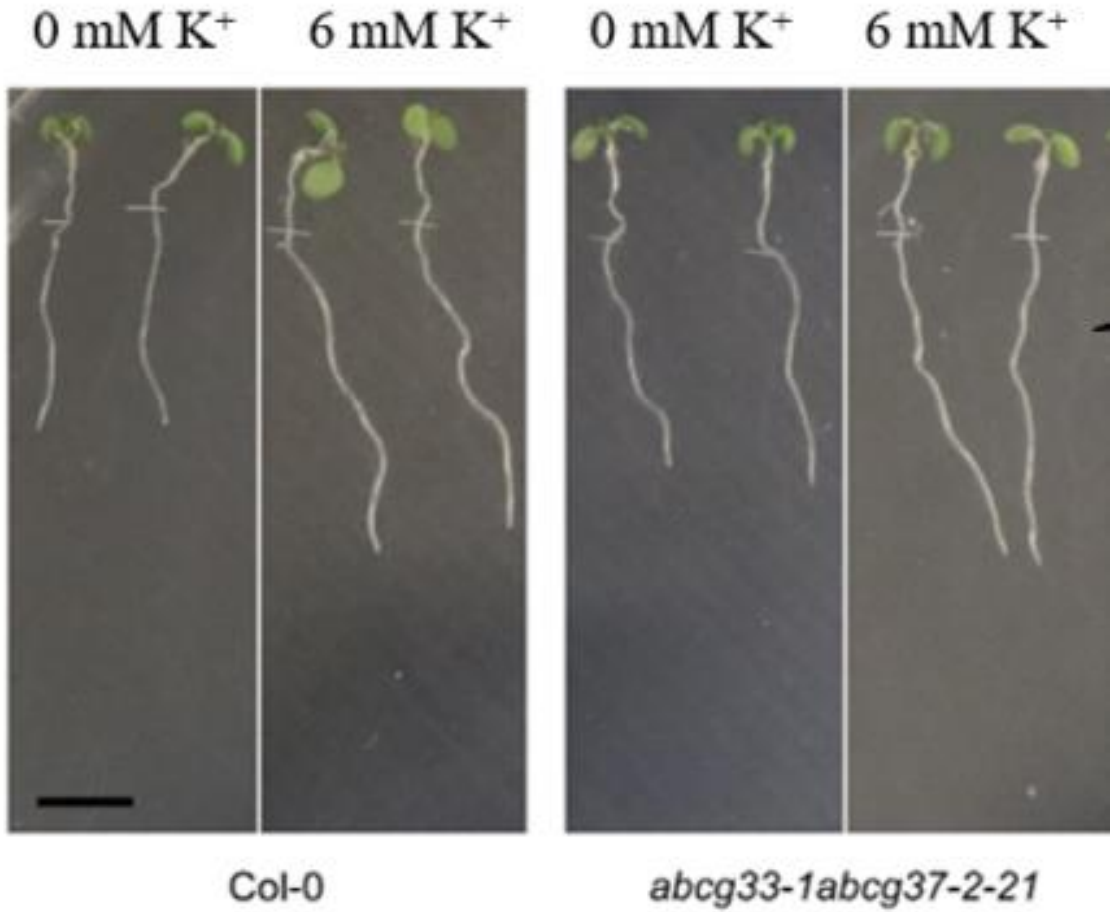


DM indicates the double mutant *abcg37-2 abcg33-1*

Iwate University 岩手大学

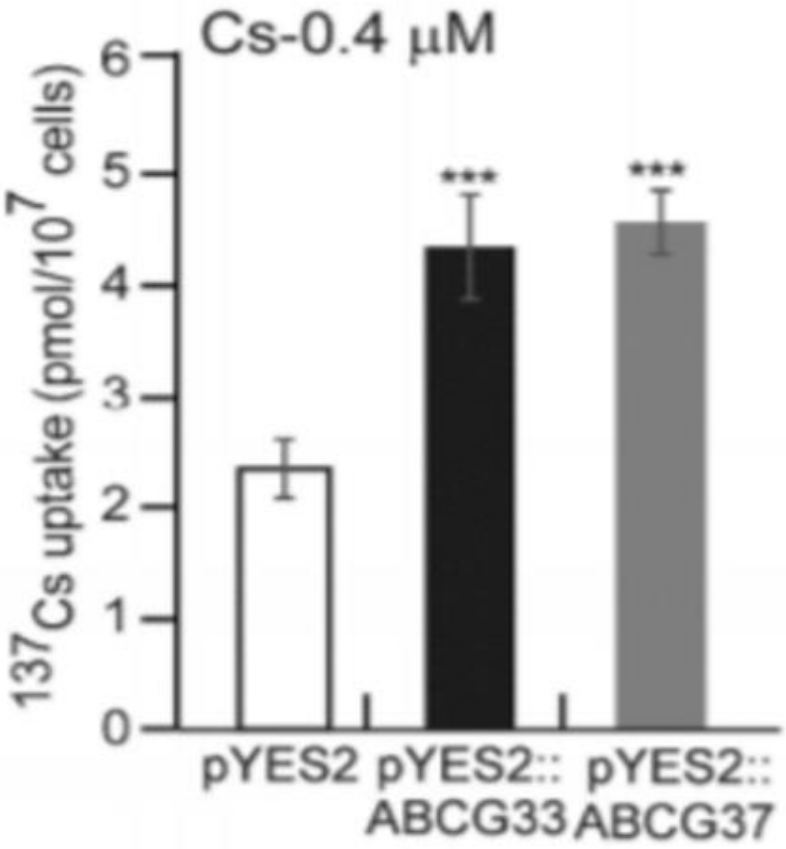
ABCG33 and ABCG37 are K^+-independent Cs^+ transporters

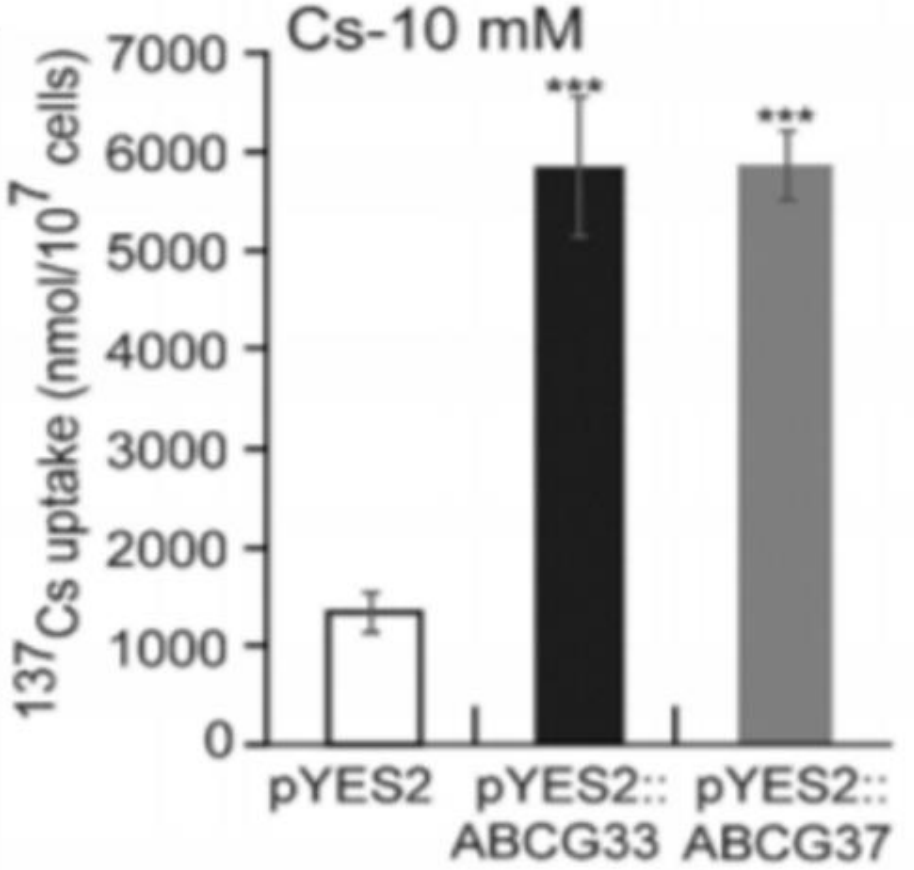




Uiwate University 岩手大学

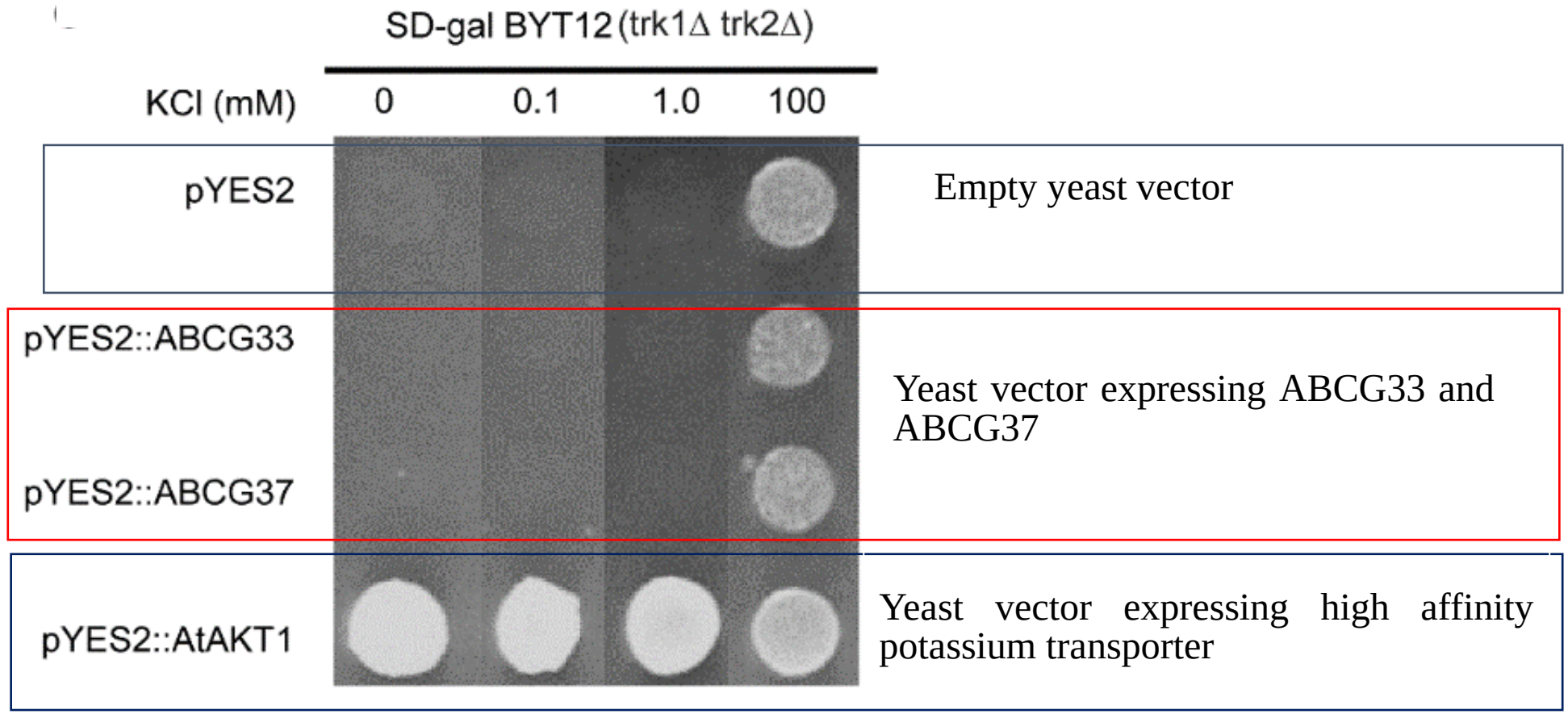
ABCG33 and ABCG37 are functional in yeast system and uptake Cs^+





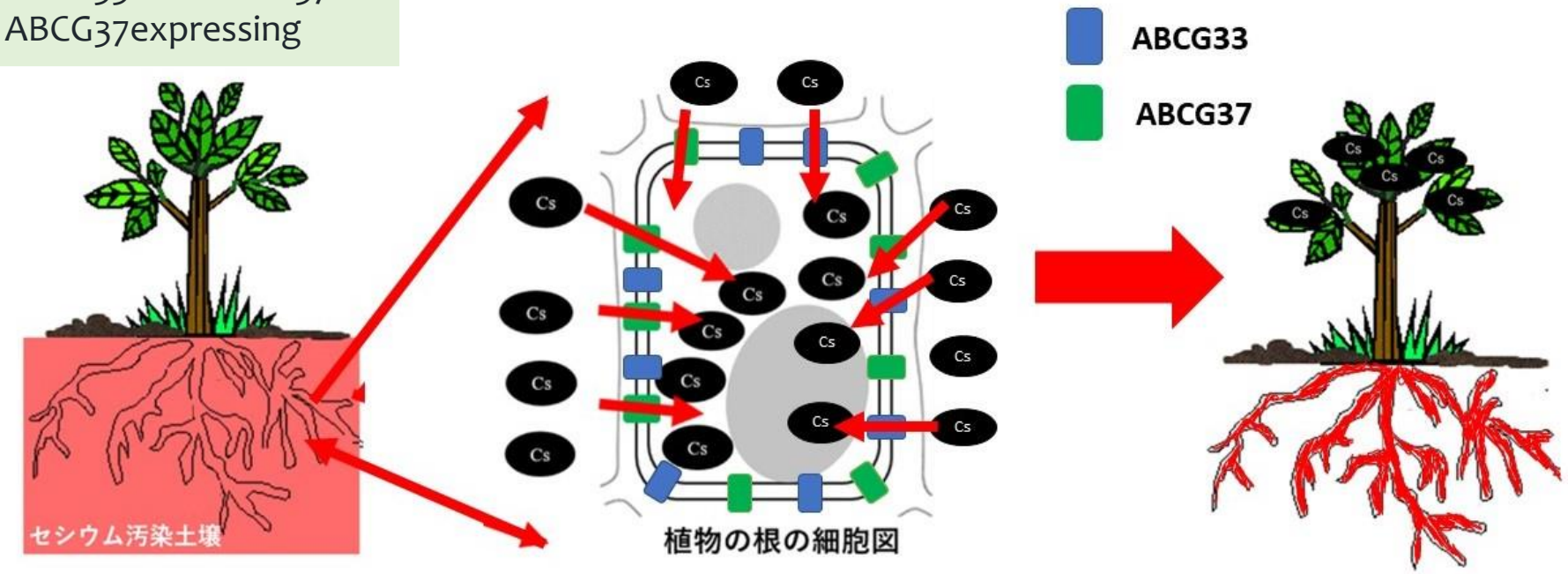
Iwate University 岩手大学

ABCG33 and ABCG37 do not uptake K^+



Iwate University 岩手大学

Plants overexpressing
ABCG33 and ABCG37
ABCG37expressing



Overexpression of ABCG33 and ABCG37 can purify cesium contaminated soil!!!

Iwate University 岩手大学

Science that inspires

A Cell Press partner journal

Molecular Plant

Table of Contents >

> View Archive

isolating genes

Wang et al.

OsCNGC9 confer enhanced chilling tolerance in rice

Wang et al.

non-canonical PAM compatibility in plants

Li et al.

Submit | Alerts | For Authors | About

Online Now

RESEARCH ARTICLE

A transceptor-channel complex couples nitrate sensing to calcium signaling in *Arabidopsis*

Wang et al.

RESEARCH ARTICLE

ATP Binding Cassette Proteins ABCG37 and ABCG33 function as potassium-independent cesium uptake carriers in Arabidopsis roots

Ashraf et al.

COMMENT

Breeding with Dominant Genic Male-Sterility Genes to Boost Crop Grain Yield in Post-Heterosis Utilization Era

Wan et al.

RESEARCH ARTICLE

The landscape of gene-CDS-haplotype diversity in rice (*Oryza sativa* L.): properties, population organization, footprints of domestication and breeding, and implications in

We got wide media coverage in Japan

International media:
AAAS's EurekAlert: tps://eurekalert.org/pub_releases/2021-02/iuj-pas021621.php

University press release:

Iwate University: https://www.iwate-u.ac.jp/english/info/news/2021/02/003918.html
University of Tokyo: https://www.a.u-tokyo.ac.jp/topics/topics_20210215-2.html

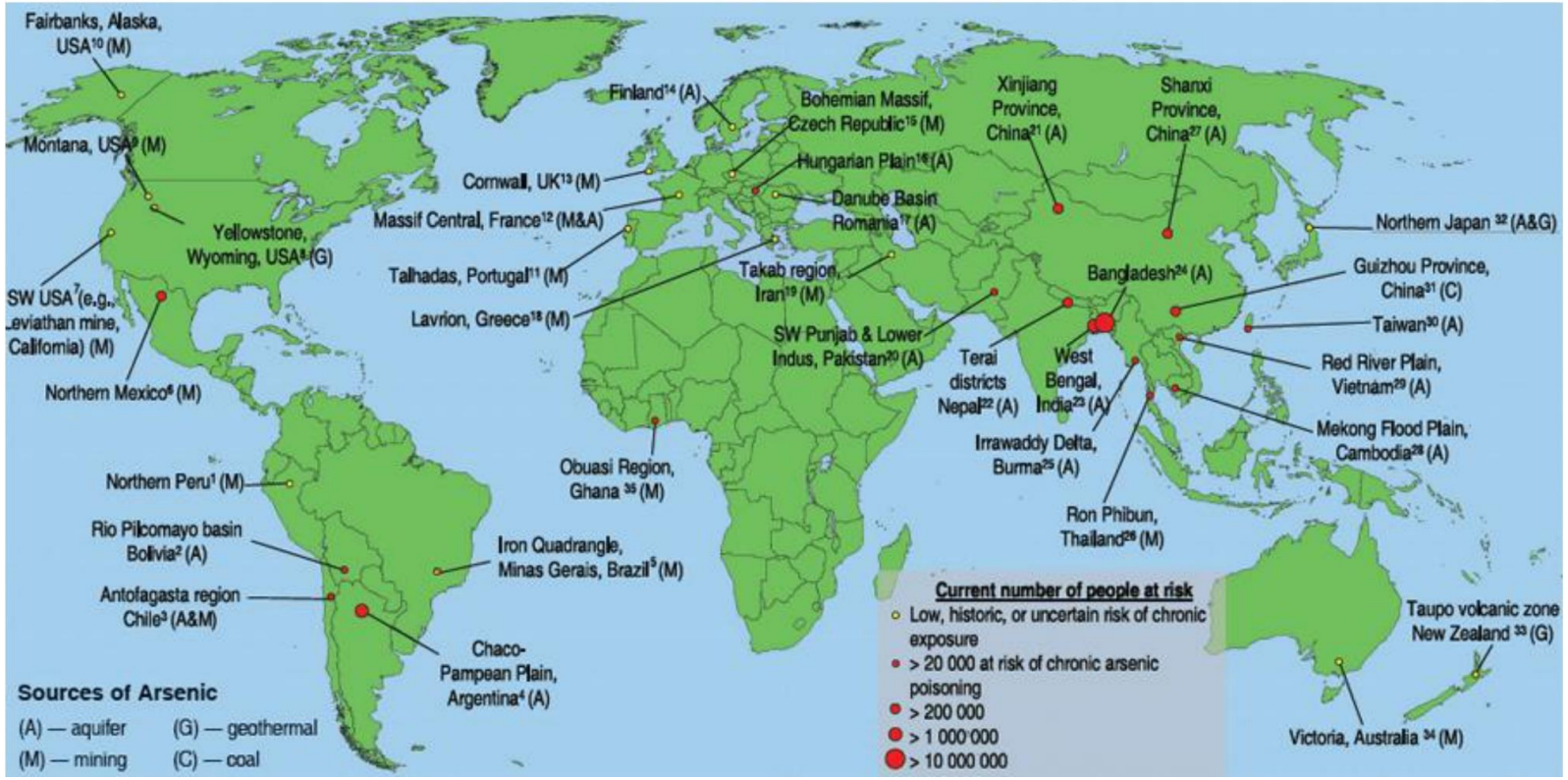
Newspaper feature:
Microsoft News Japan: http://a.msn.com/01/ja-jp/BB1dJ5Bv?ocid=sli

FNN Prime Online: https://www.fnn.jp/articles/-/145076

Iwate Nippo: Attached image from the printed newspaper (https://www.iwate-np.co.jp/article/2021/2/17/92217)
TV channel coverage:
NHK: https://www3.nhk.or.jp/lnews/morioka/20210216/6040009961.html
Iwate Menkoi TV: https://youtu.be/1q9z3hMmsKM

Iwate University 岩手大学

Global arsenic distribution



- 140 million people in 50 countries have been drinking water containing arsenic at levels above the WHO provisional guideline value of 10 µg/L

Iwate University 岩手大学

Key Facts

•Arsenic is naturally present at high levels in the groundwater of a number of countries.

- Arsenic is highly toxic in its inorganic form.

•Contaminated water used for drinking, food preparation and irrigation of food crops poses the greatest threat to public health from arsenic.

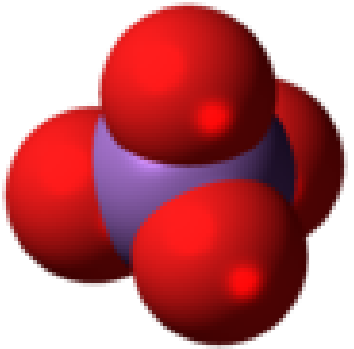
•Long-term exposure to arsenic from drinking-water and food can cause cancer and skin lesions. It has also been associated with cardiovascular disease and diabetes.

https://www.who.int/news-room/fact-sheets/detail/arsenic

Inorganic Arsenic exists in two forms:

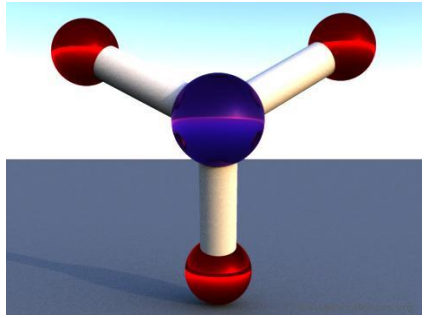
1) Fully oxidized pentavalent form: Arsenate

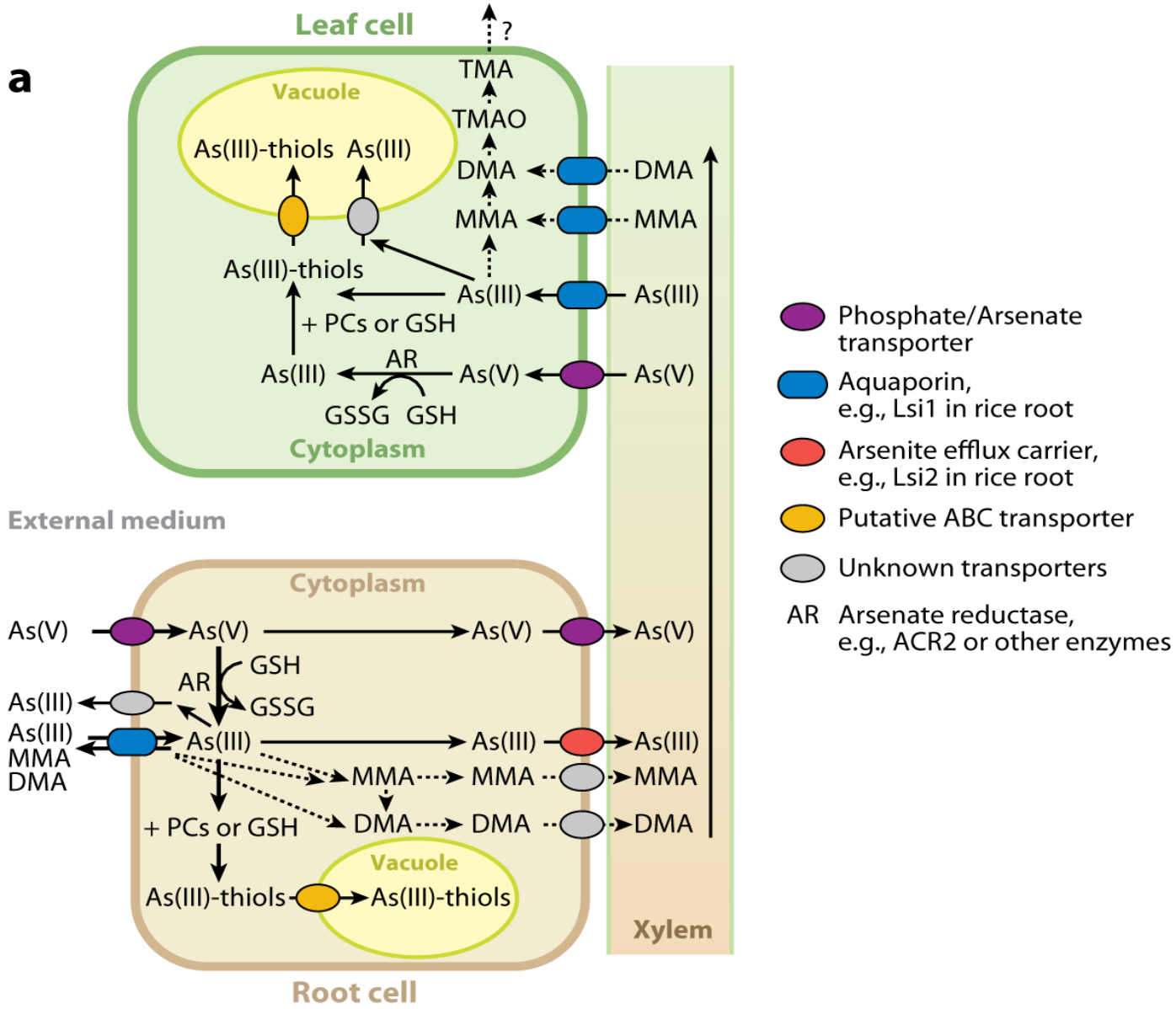
AsO_4^{3-} Phosphate analogue



2) Reduced trivalent form: Arsenite

AsO_3^{3-}





- Arsenate is transported by phosphate transporters
- Arsenite uptake is mediated by aquaporins
- Arsenite efflux carrier is unknown

Iwate University 岩手大学

- Only one protein LSi2 functions in cellular effflux of arsenite
  LSi2 is only present in rice, not in other higher plants

Possibility and Question:

- There must be other proteins that may function to facilitate cellular efflux of arsenite
- What are the proteins???

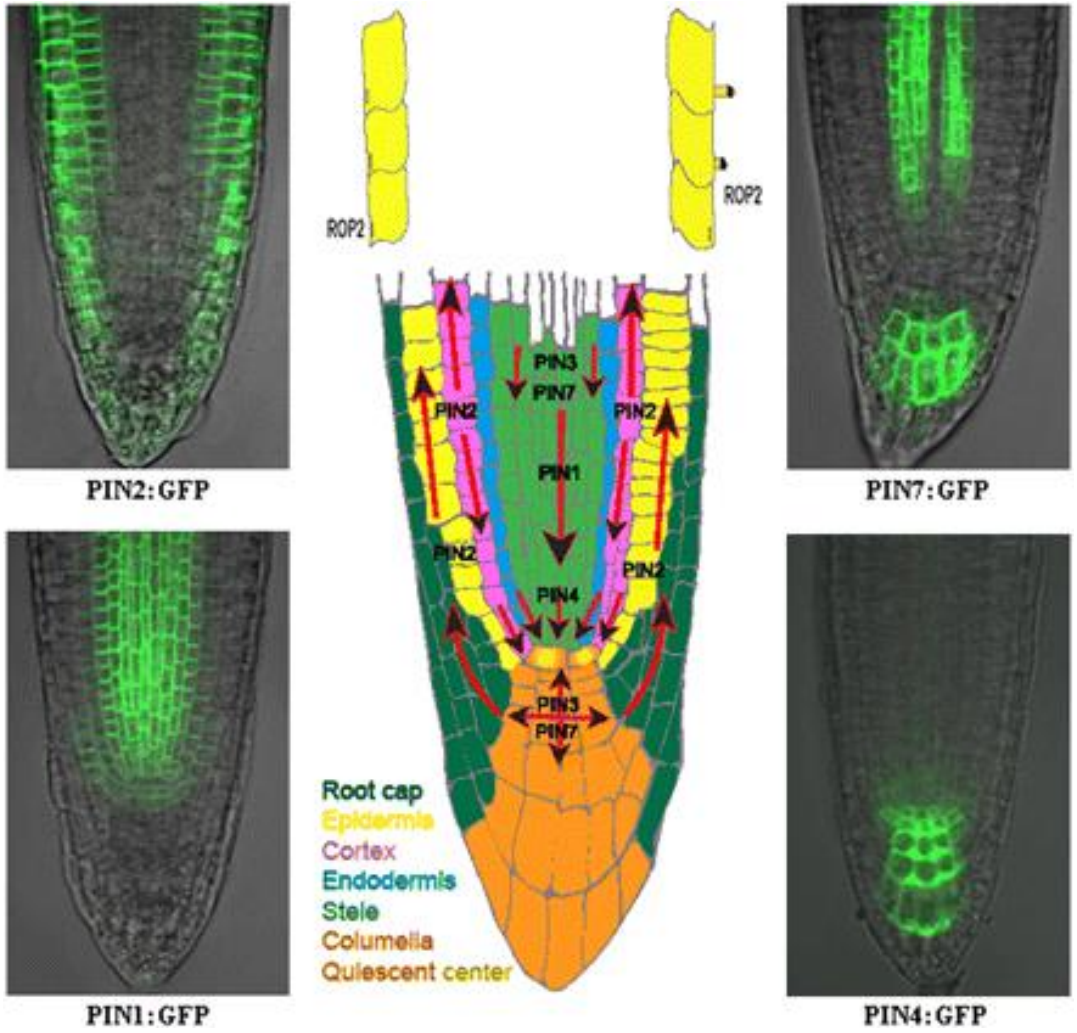
EIR1, *a highly conserved plant gene family with similarities to bacterial transporters*

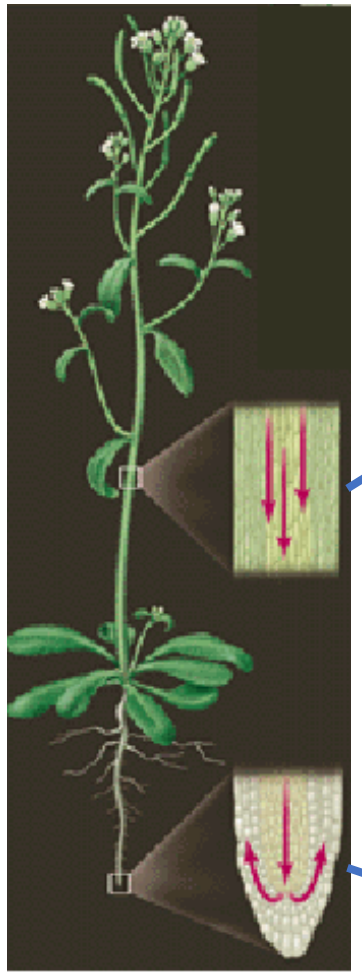
Particularly noteworthy is the similarity of EIR1 to the class of efflux carriers that remove toxic compounds from the interior of the cell. For example, *E. coli arsB* (P52146) represents a part of the arsenic efflux system (Diorio et al. 1995). *sbmA* (X54153), another integral membrane protein of *E. coli*, has been shown to be necessary for uptake of the antibiotic Microcin 25 (Salomon and Farias 1995). Portions of EIR1 show 35%–40% similarity to these proteins. Our finding that the amino and the carboxyl terminus of EIR1 exhibit similarities to the

Luschnig et al., 1998; Genes &Dev

Portions of EIR1 show 35%-40% homology to bacterial arsenite transporter *arsB*

Auxin efflux carrier/PIN family proteins transport plant hormone auxin





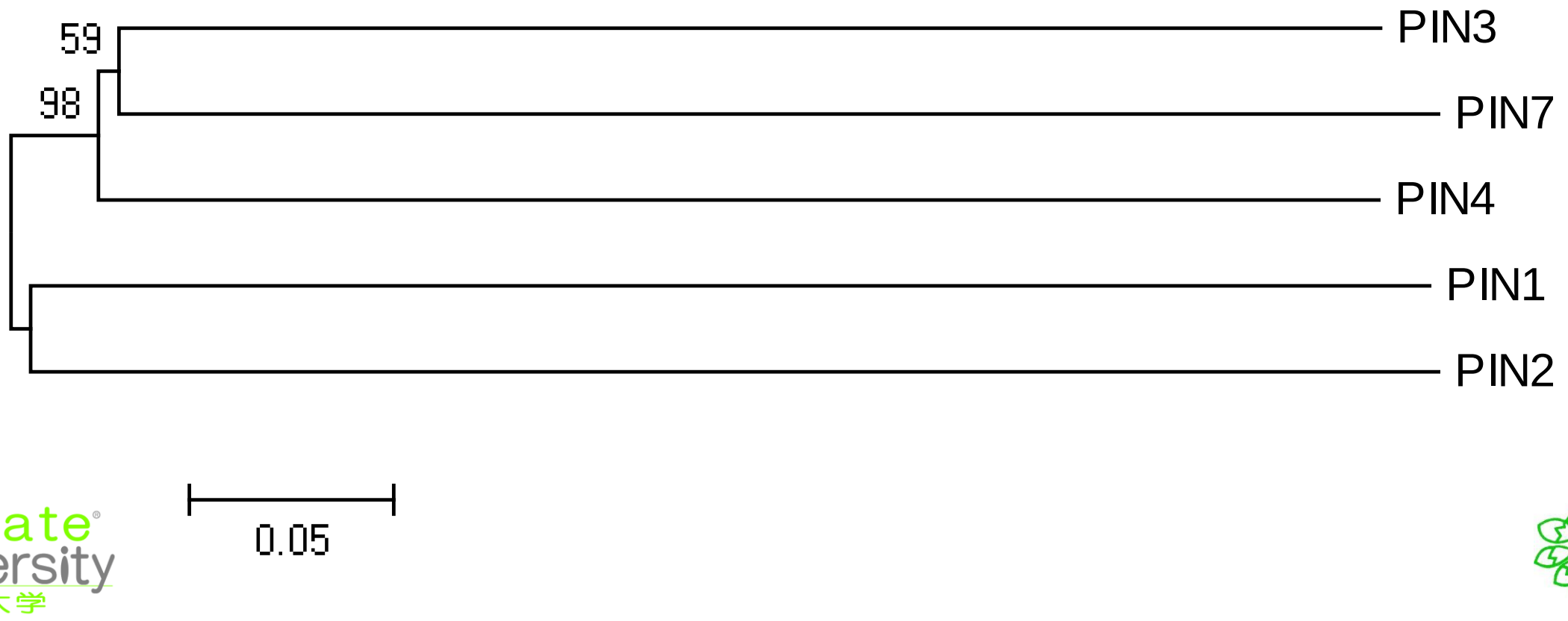
Acropetal transport
(rootward transport)

Basipetal transport
(shootward transport)

Jones A, Science (1998)

Iwate University 岩手大学

Cladogram of plasma membrane residing PIN proteins



Iwate University 岩手大学

Identity matrix of *Saccharomyces cerevisiae* ACR3 (SsACR3), *Pteris vittata* ACR3 (PvACR3) and *Escherichia coli* arsenite transporter (arsB), *Arabidopsis thaliana* PINs (AtPIN1, AtPIN2, AtPIN3).

	SsACR3	PvACR3	arsB	AtPIN2	AtPIN1	AtPIN3
SsACR3	100.00	40.16	8.22	16.67	18.01	17.28
PvACR3	40.16	100.00	13.99	17.65	18.45	18.13
arsB	8.22	13.99	100.00	24.51	24.44	24.86
AtPIN2	16.67	17.65	24.51	100.00	62.88	60.88
AtPIN1	18.01	18.45	24.44	62.88	100.00	65.49
AtPIN3	17.28	18.13	24.86	60.88	65.49	100.00

Iwate University 岩手大学

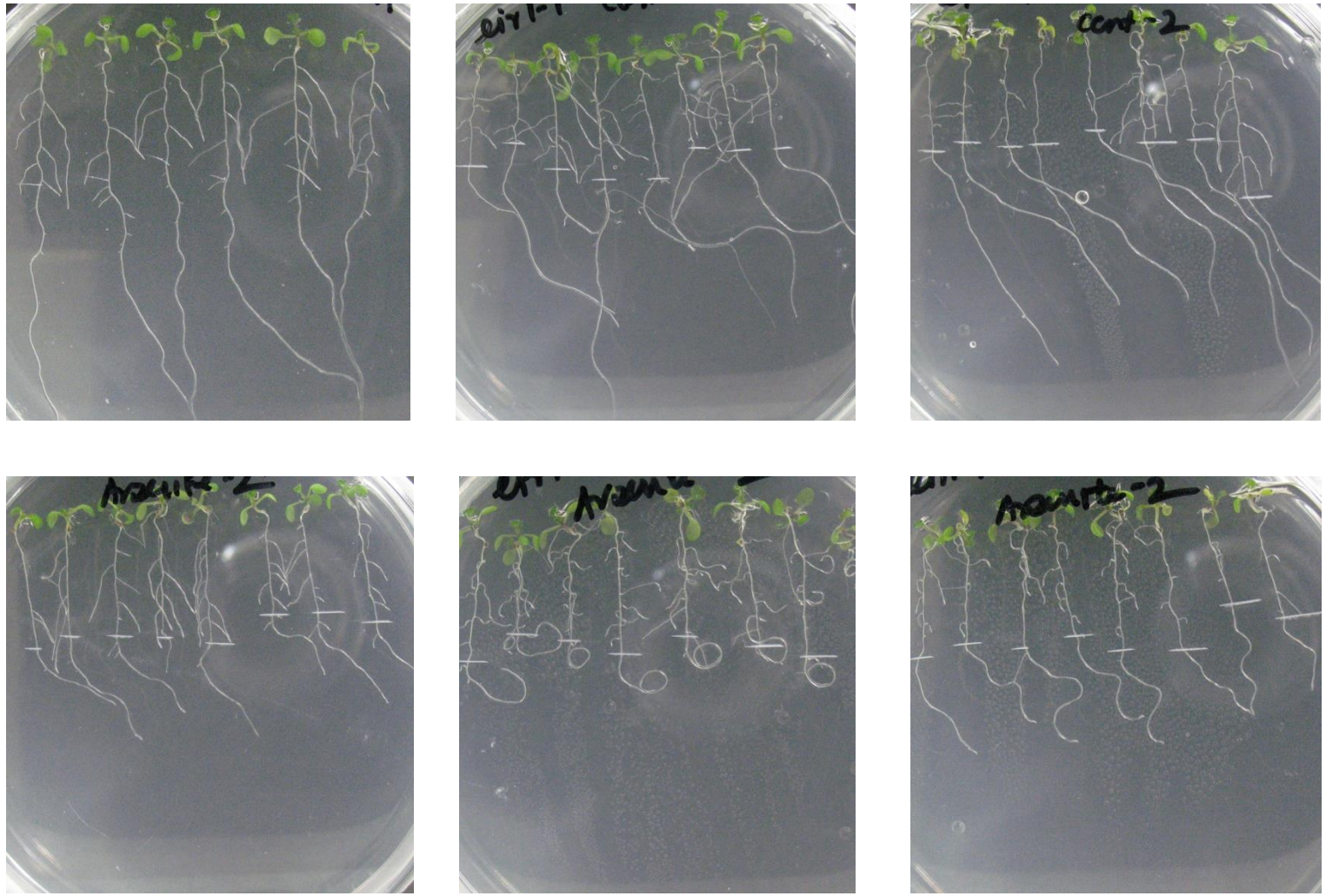
Identity matrix of OSLsi2 and arsenite transporters

	ScACR3	PvACR3	arsB	OsLsi2
ScACR3	100.00	40.87	10.89	9.27
PvACR3	40.87	100.00	10.19	7.43
arsB	10.89	10.19	100.00	14.29
OsLsi2	9.27	7.43	14.29	100.00

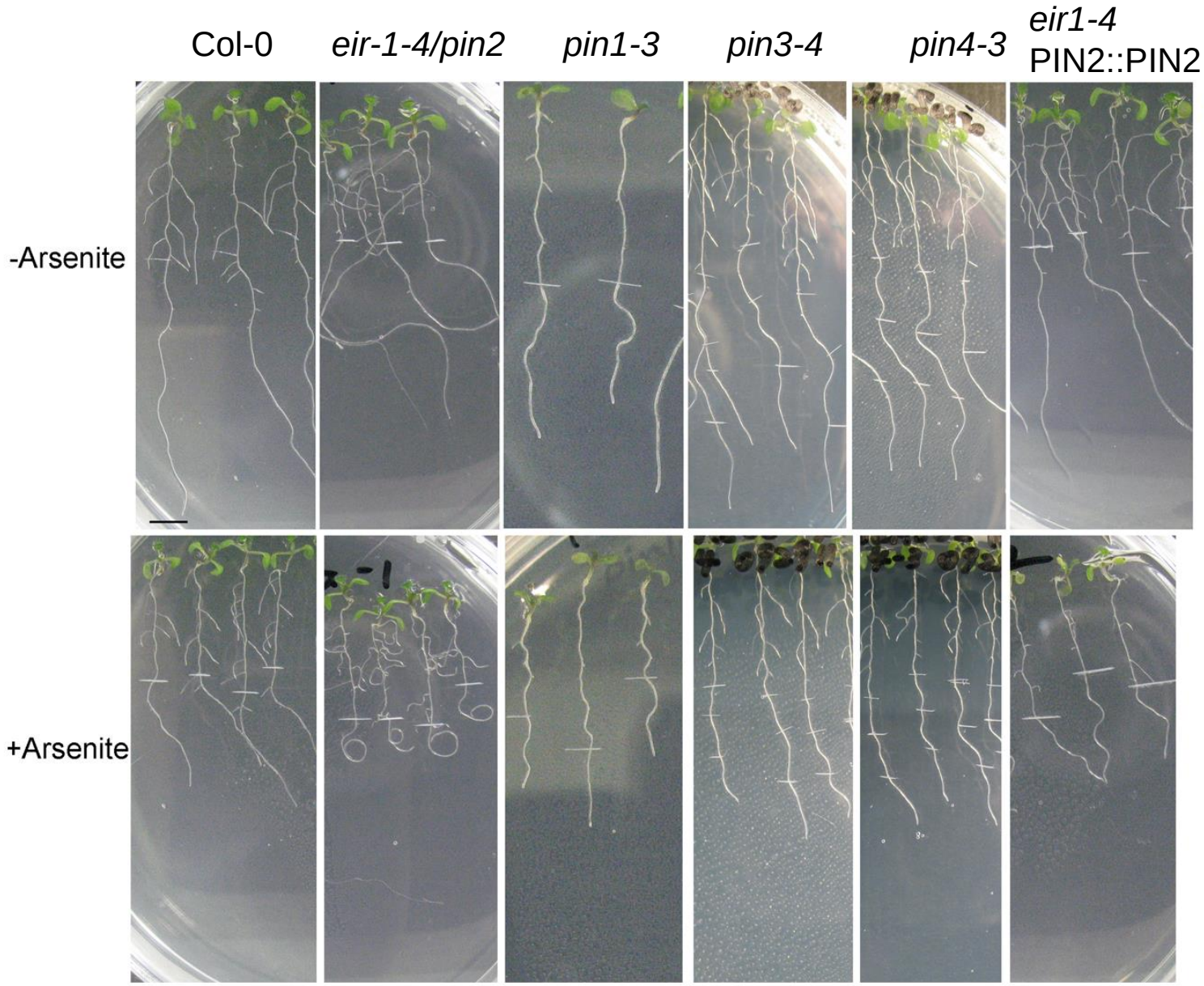
Iwate University 岩手大学

PIN2 mutant (*pin2*) shows hypersensitive response to arsenite

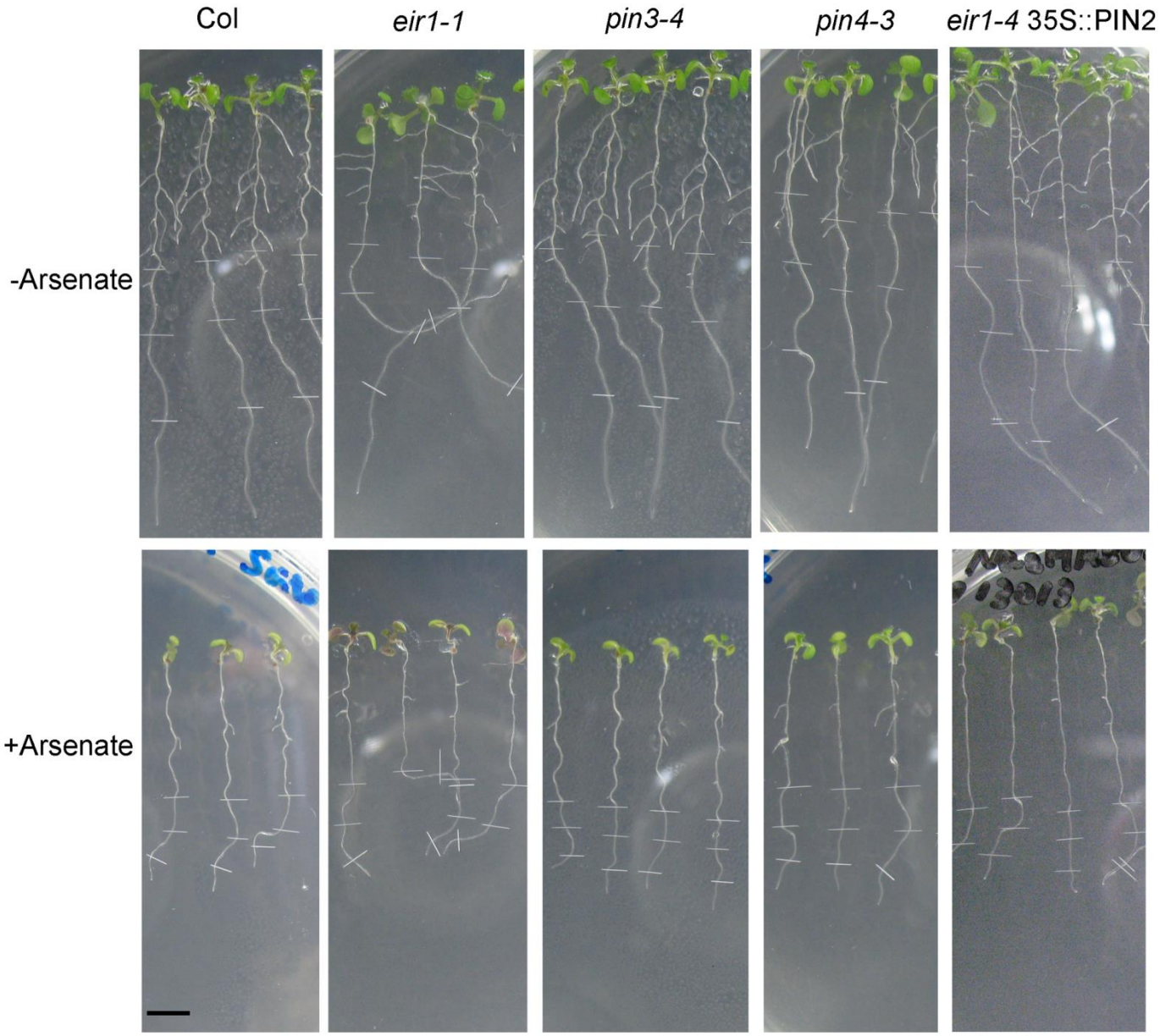
Col-0 | *eir1-4/pin2* | *eir1-4::35SPIN2*



Other PIN mutants show wild-type like response to arsenite



pin2 mutant responds to arsenate like wild-type



Iwate University 岩手大学

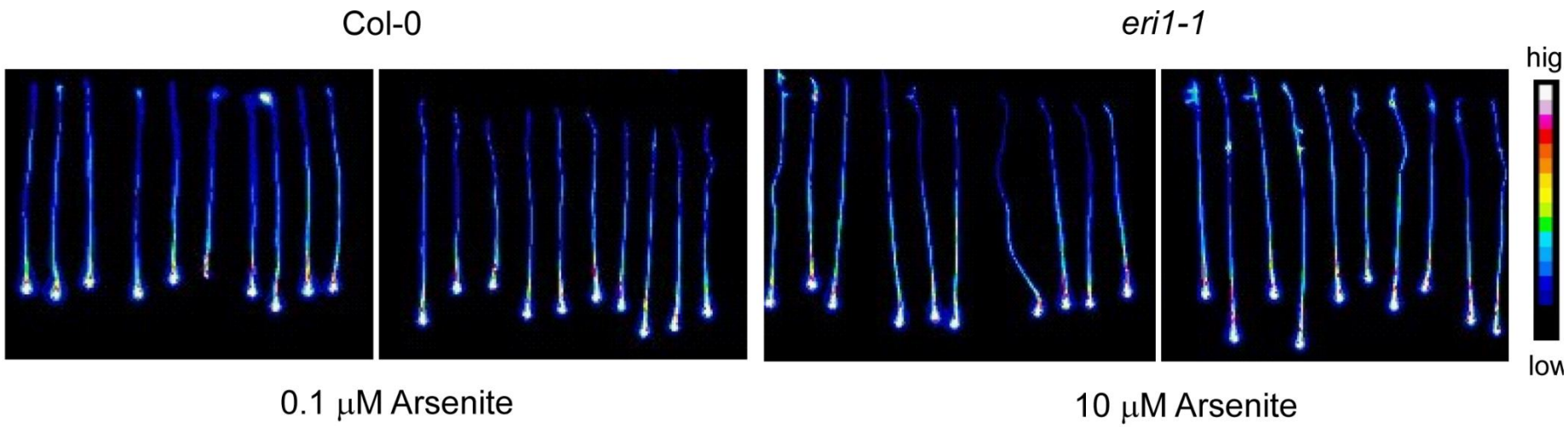
Summary

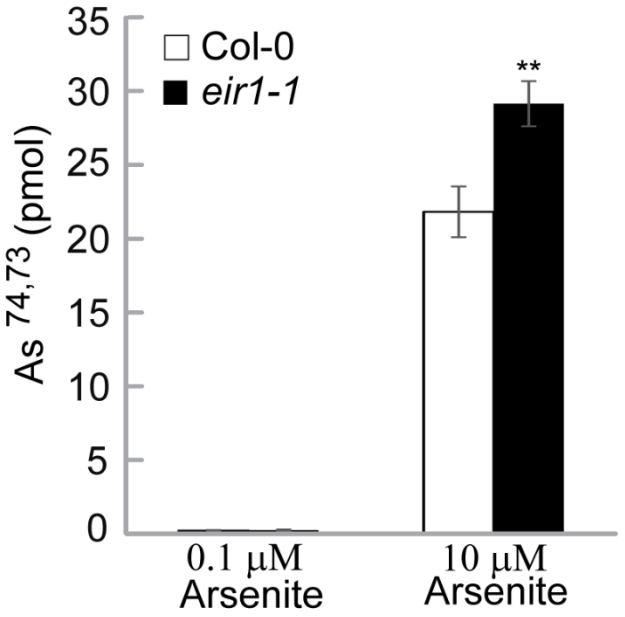
- Auxin efflux carrier PIN2 regulates root arsenite but not arsentae response in Arabidopsis

- Although all the PINs show similar homology to bacterial arsenite transporter arsB, only PIN2 is targeted by arsenite

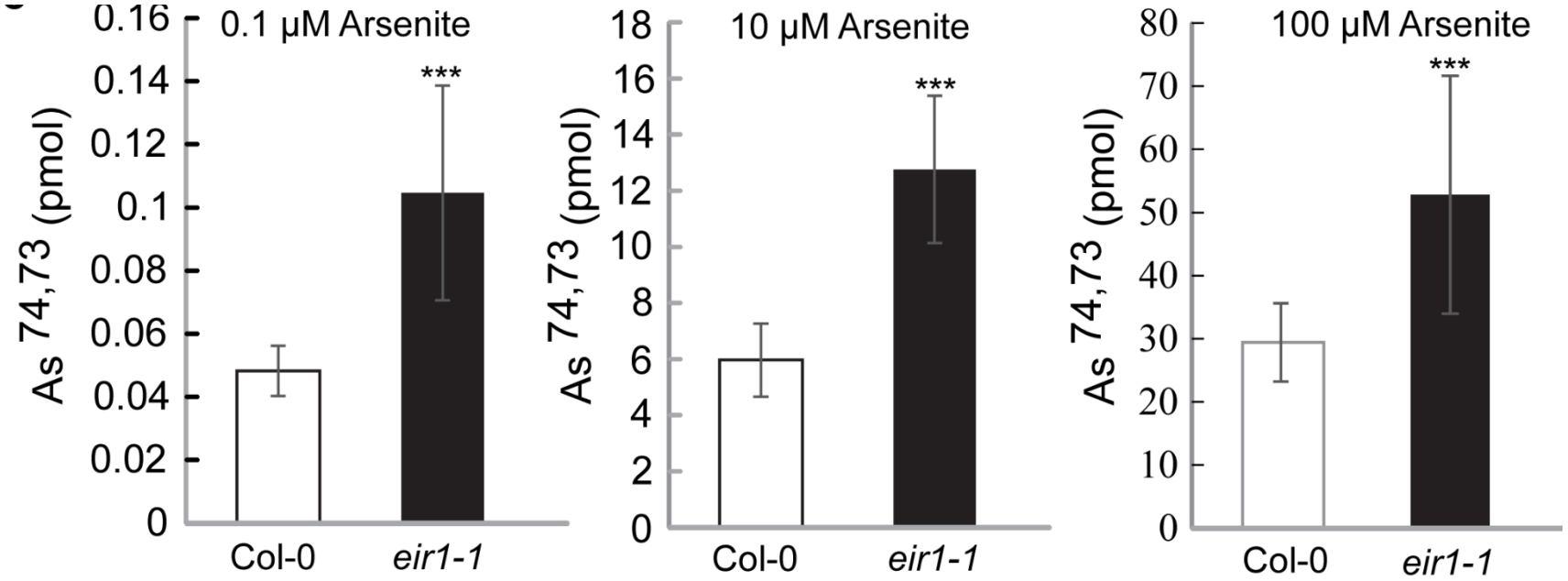
Iwate University 岩手大学

Arsenite transport is reduced in *pin2* mutant





More Arsenite is accumulated in *pin2* mutant

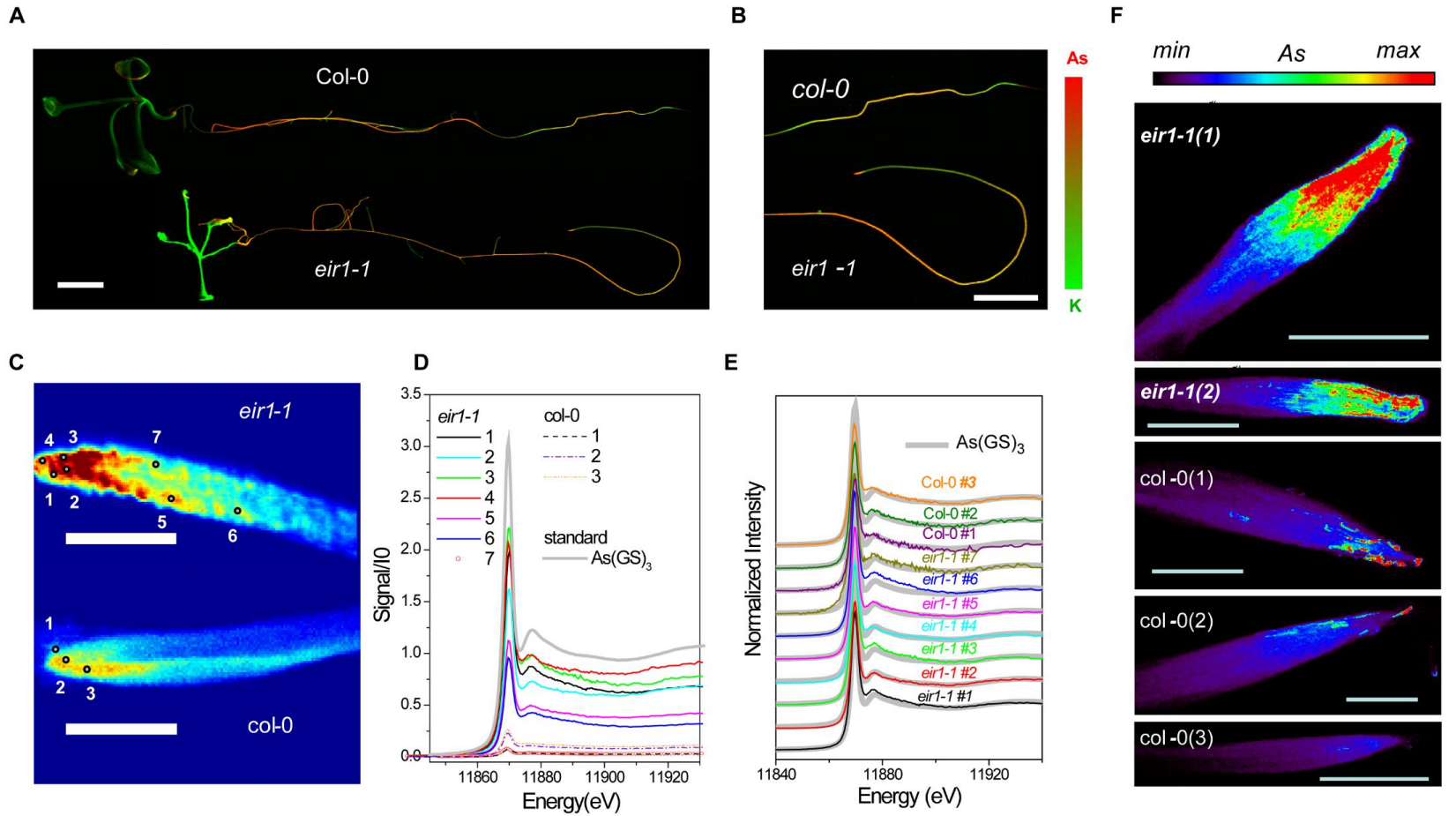


Iwate University 岩手大学

Elemental mapping by Synchotron Imaging

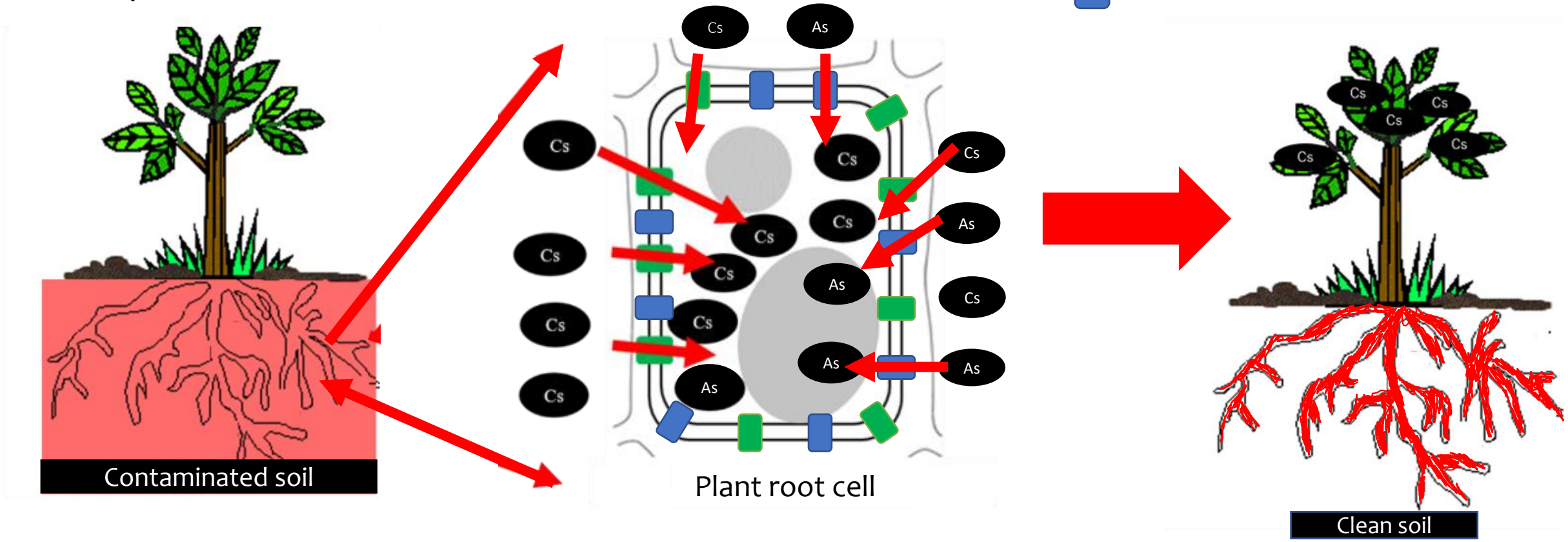
Iwate University
岩手大学

More Arsenite is accumulated in *pin2* mutant



Iwate University 岩手大学

Plants over expressing specific metal transporters



Outline

Plant Communications
Volume 1, Issue 3, 11 May 2020, 100009

Research Article

PIN FORMED 2 Modulates the Transport of Arsenite in *Arabidopsis thaliana*

Mohammad Arif Ashraf [1, 11], Kana Umetsu [2, 11], Olena Ponomarenko [3, 11], Michiko Saito [2], Mohammad Aslam [2], Olga Antipova [4], Natalia Dolgova [3], Cheyenne D. Kiani [3, 12, 13], Susan Nehzati [3], Keitaro Tanoi [5], Katsuyuki Minegishi [6], Kotaro Nagatsu [6], Takehiro Kamiya [7], Toru Fujiwara [7], Christian Luschnig [8], Karen Tanino [9], Ingrid Pickering [3], Graham N. George [3], Abidur Rahman [1, 2, 10]

https://doi.org/10.1016/j.xplc.2019.100009

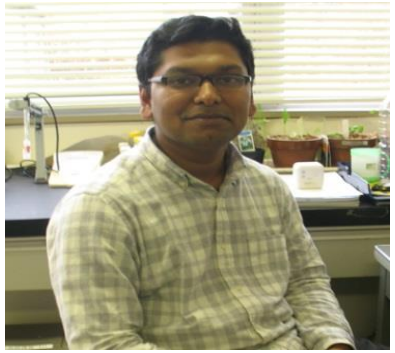
Part of special issue:

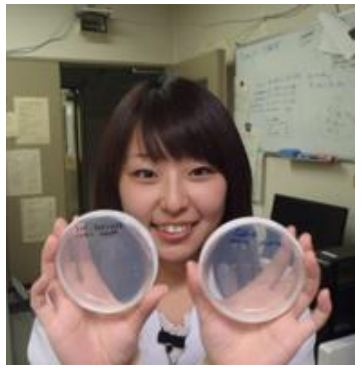
Focus Issue on Plant Hormones (Organizing Editors: Steven Smith, Jan Hejátko, Yonghong Wang, Daoxin Xie)

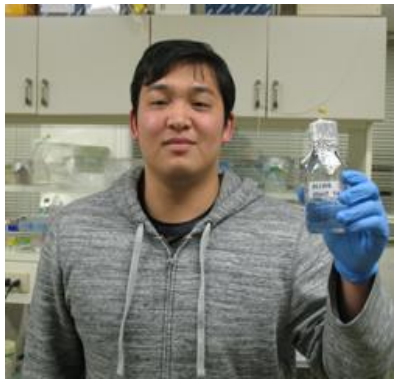
Edited by Steven Smith, Jan Hejátko, Yonghong Wang, Daoxin Xie

Acknowledgements

Abidur lab- October-2020







Collaborators

Keitaro Tanoi
Toru Fujiwara
University of Tokyo

Graham George
Karen Tanino
University of Saskatchewan

科研費 KAKENHI

Iwate University 岩手大学

岩手大学 次世代アグリイノベーション研究センター Agri - Innovation Center , Iwate University

JSPS

University of Saskatchewan

UGAS THE UNITED GRADUATE SCHOOL OF AGRICULTURAL SCIENCES, IWATE UNIVERSITY