Influence of Potassium Solubilizing Bacteria on Growth and Radiocesium Accumulation of *Brassica rapa* L. var. *perviridis* grown in Cs-Contaminated Fukushima Soils

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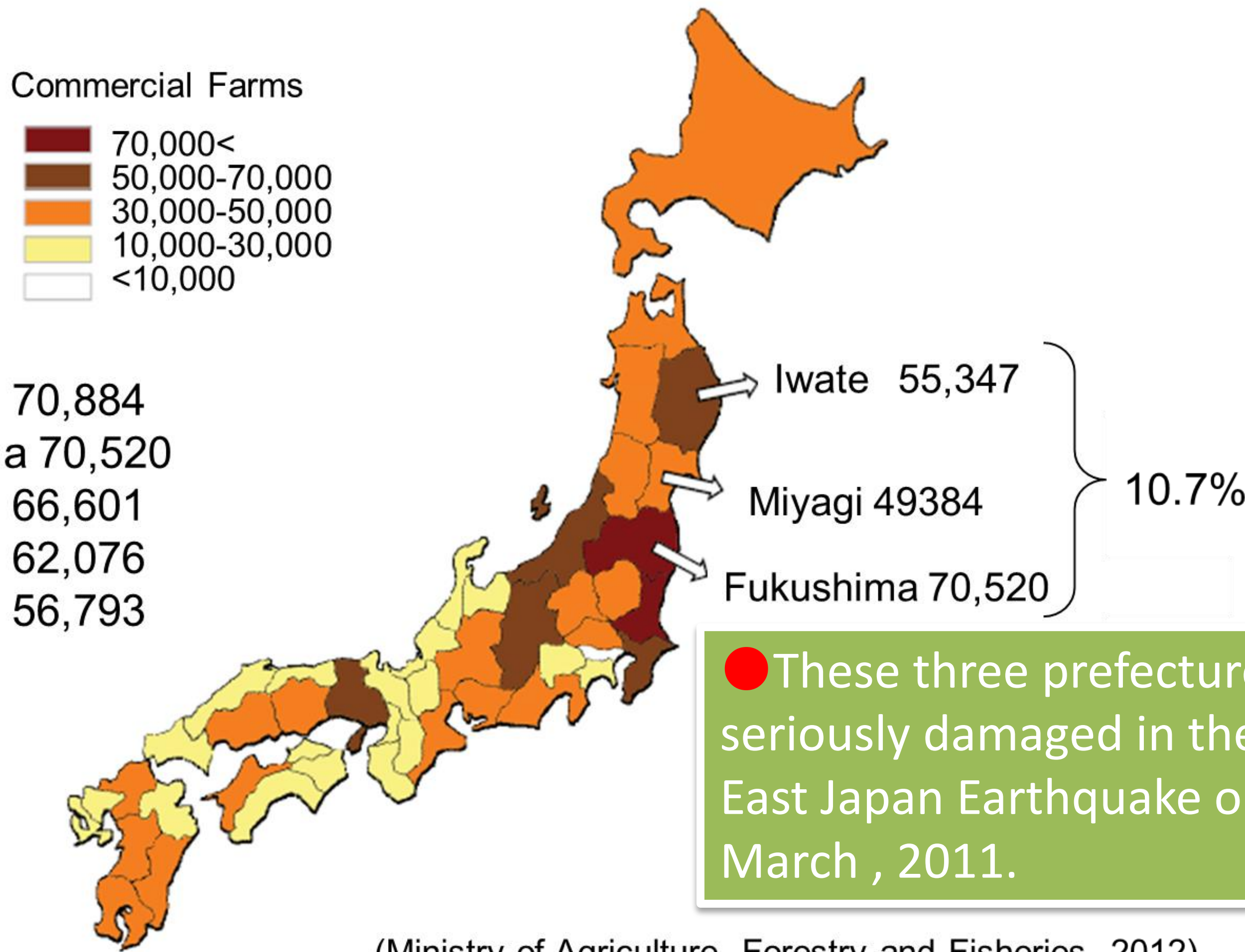
A study conducted under the supervision of Dr. T. Yokoyama during the FNCA-MEXT Fellowship Program (October 2013-March 2014) Tokyo University of Agriculture and Technology, Japan

Technical Workshop on “Remediation of Radioactive Contamination in Agriculture"
17-18 October 2016 Vienna, Austria.

Agriculture in Fukushima

1. Ibaraki 70,884
2. Fukushima 70,520
3. Niigata 66,601
4. Nagano 62,076
5. Hyogo 56,793



(Ministry of Agriculture, Forestry and Fisheries, 2012)

● These three prefectures were seriously damaged in the Great East Japan Earthquake on 11, March , 2011.

- commercial farms in Fukushima are 70,520
- 2nd among 47 prefectures in Japan

Limited agricultural knowledge to find the best way to cope with the difficult situations on March 2011.

Deep plowing

Removal of surface soil

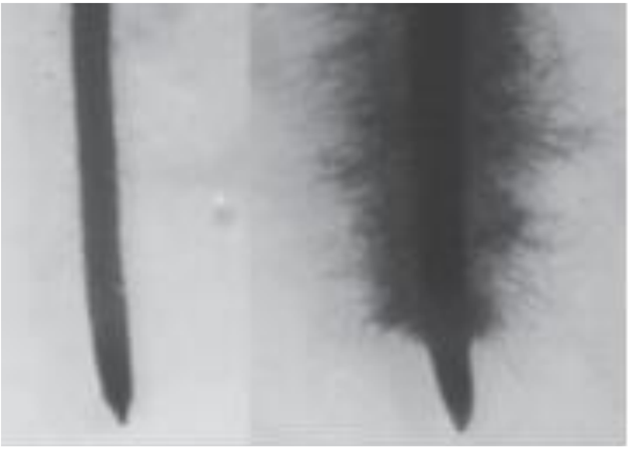
The deep plowing and removal of surface soil are very effective to reduce contamination of radioactive Cs from contaminated soils.

- Deep plowing: 50% reduction of radioactive Cs in a surface soil.
- Removal of surface soil : 75% reduction of radioactive Cs from a contaminated soil.

Many researchers belonging to different universities came together to help solve the problem.

Yokoyama, 2013

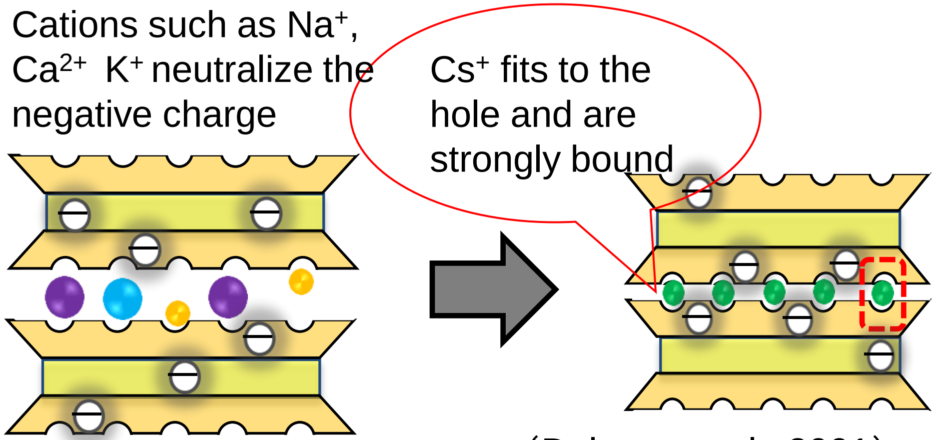
Development of bioremediation of radioactive Cs from contaminated soils using a plant-microbe interaction



Plant Growth Promoting Rhizobacteria

- Growth enhancement (root growth & activity) , which leads to maximum radioactive Cs uptake to the plant

- Search for potassium solubilizing bacteria, which produce acidic materials and those can open the inner-sphere of clay minerals and release Cs



(Delvaux et al., 2001)

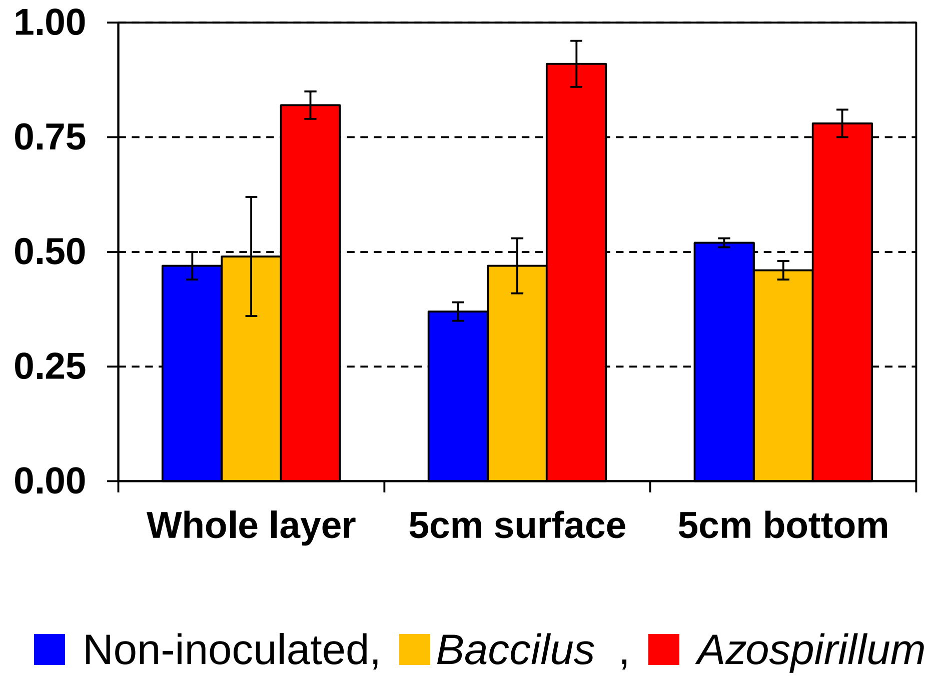
Enables effective removal of movable (mobile) Cs from the soil!

Yokoyama, 2013

Preliminary results in TUAT

(TUAT, 30th Nov. 2011)

Brassica rapa var. perviridis (Komatsuna)



(Salem et al. 2013)

Removal rate

Bacillus PGPR : 5% of Cs

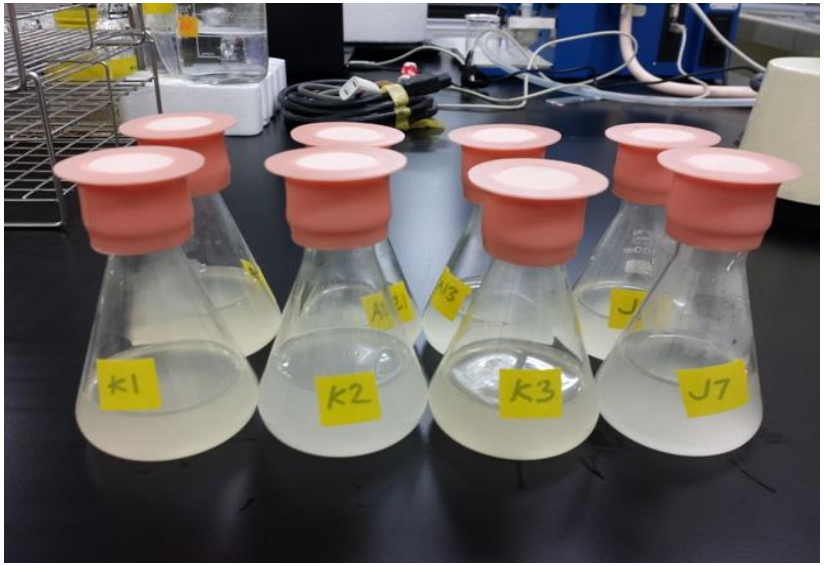
Azospirillum PGPR: 9% of Cs

● Transfer factor = (Bq kg^{-1}plant) / (Bq kg^{-1} kg soil)

Yokoyama, 2013

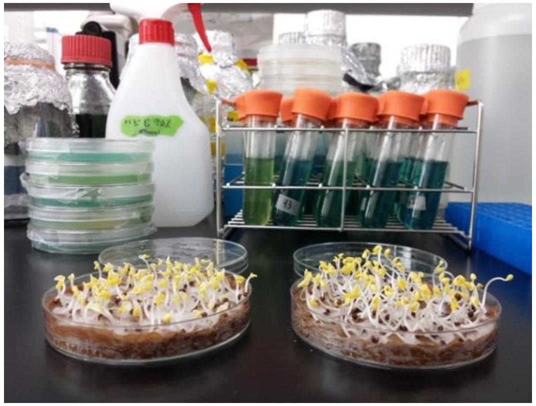
The Experiment

- Exploration of KSB and its influence on the growth and radiocesium accumulation in Komatsuna



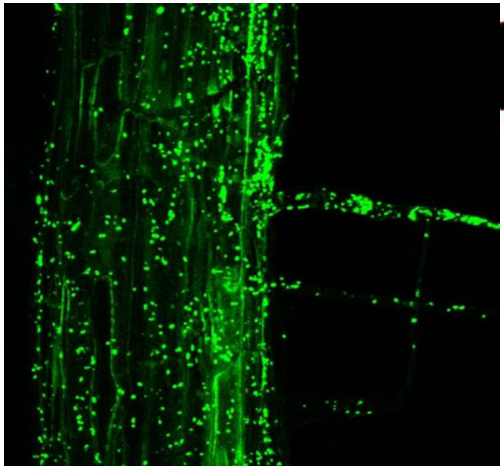
Principles

- manipulations of the growth media ionic concentrations and ratios (e.g. K^+: Cs^+)
- increasing plant biomass in the contaminated soils per unit area
- pgpr's can increase plant biomass and uptake of metals and radionuclides
- practical application to manage or if possible enhanced phyto-transfer in contaminated soils.

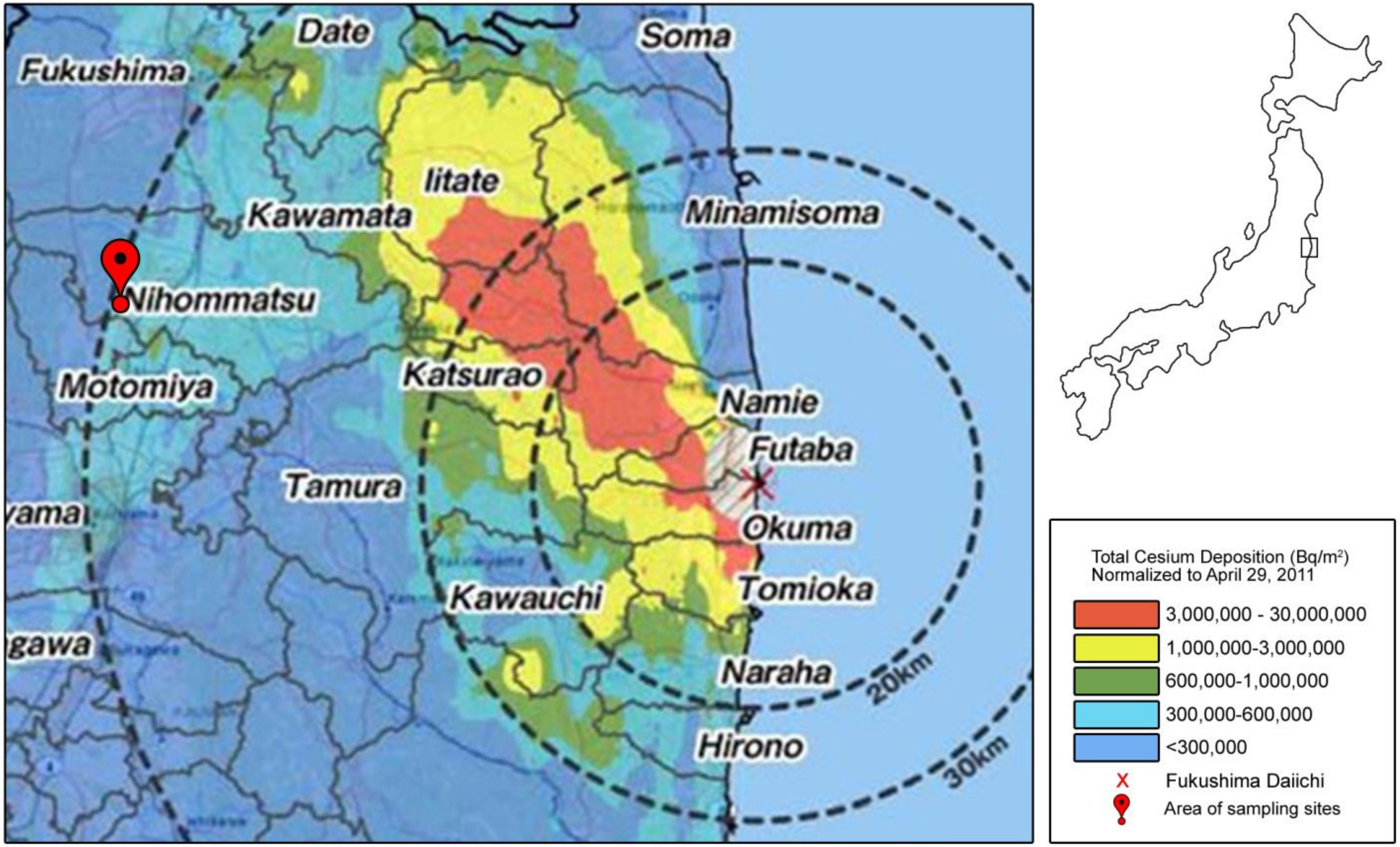


Potassium solubilizing bacteria (KSB)

- rhizospheric microorganism which usually applied as cheap biofertilizer to solubilize the insoluble potassium (K) to soluble forms of K^+
- main mechanism of KSB is acidolysis, chelation, exchange reactions, complexolysis and production of organic acid
- to solubilize fixed K in minerals muscovite, orthoclase, biotite, feldspar, illite, and mica



Sampling Sites



Physico-chemical properties of Soils

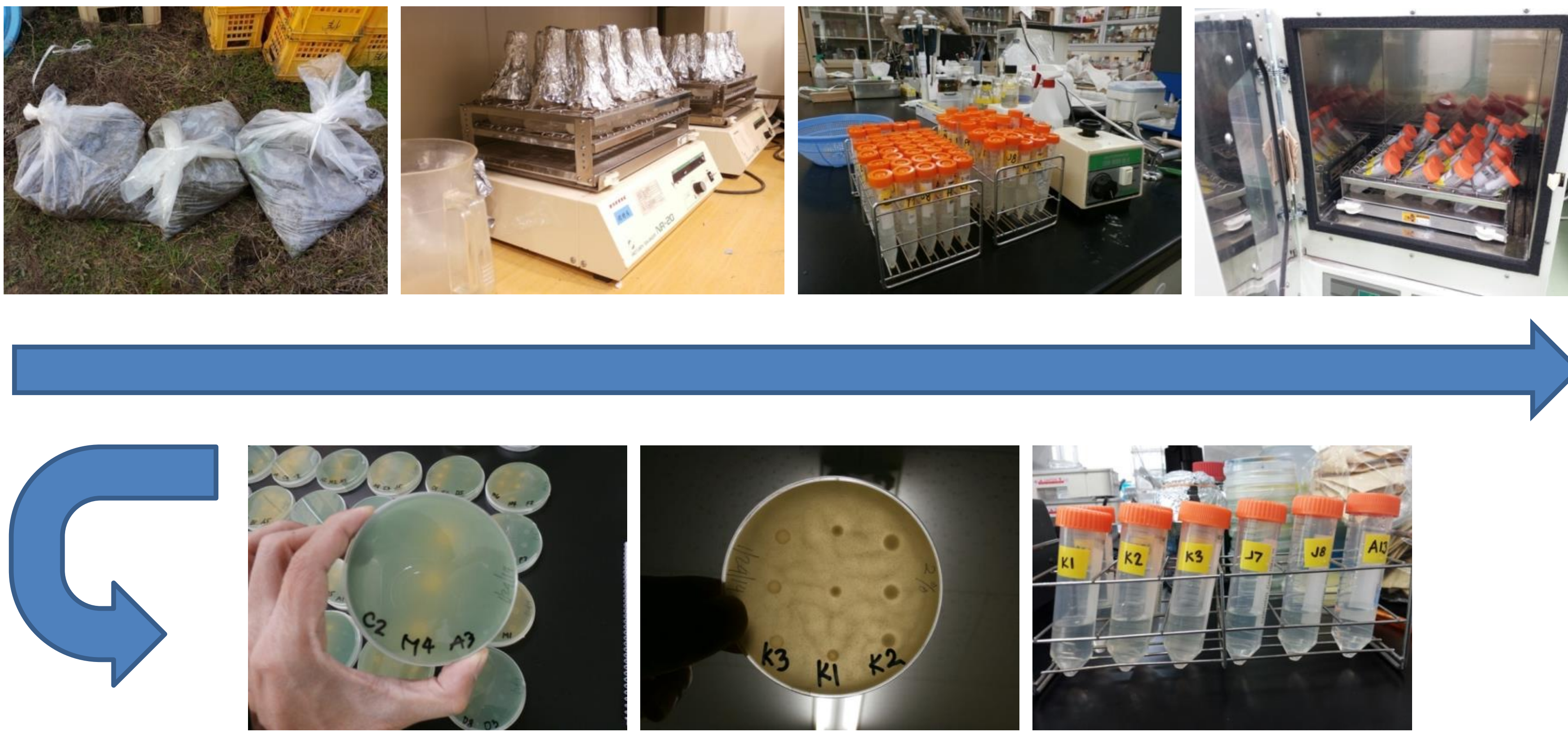
Parameters	Soil 1 Tabaco Field (MIYANOIRI)	Soil 2 Soybean Field (TAKANISHI)	Soil 3 Apple Orchard (TBK)
Parent Material	Alluvial	Alluvial	Volcanic
Textural class	Sandy clay loam	Clay	Sandy loam
Sand (%)	66	32	nd
Silt (%)	6	21	nd
Clay (%)	29	47	nd
pH	5.85	5.65	5.42
Organic Matter (%)	3.9	4.13	2.8
CEC ($cmol_{(+)}$ kg^{-1})	10	22	24
Exchangeable K (cmol kg^{-1})	1.20	1.05	1.15
Exchangeable Cs(Bq kg^{-1})	79	*	107
Cs activity (Bq kg^{-1})	1322	2464	3190

** -below detection limit*   *nd – not determined*

To convert K cmol (+)/kg x 390 = mg/kg

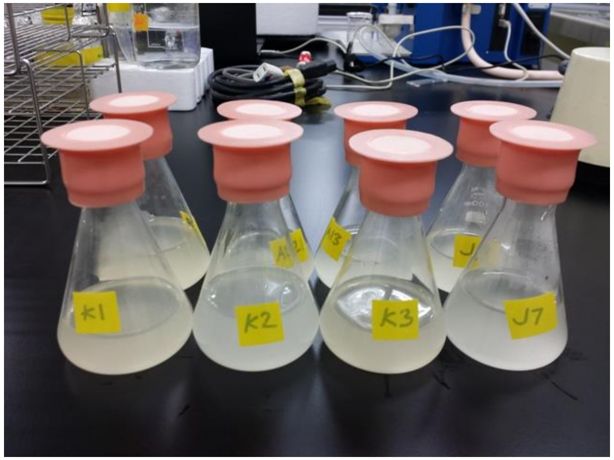
Methodology

- Isolation of KSB



Characterization of KSB

- Molecular characterization
- 16s RNA gene sequencing
- Quantitative K solubilization



- Characteristic of 5 selected KSB isolates

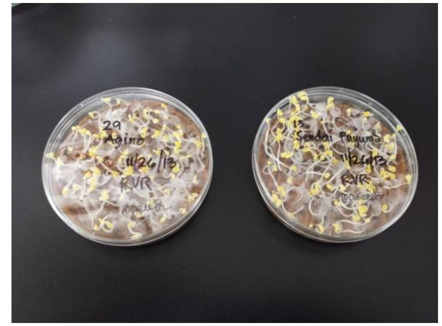
Bacterial Isolates	D/d (ratio)	K – Solubilization ($\mu g ml^{-1}$)	Isolates Genera
J7	1.17	32^{b}	*Bacillus megaterium*
J8	1.02	29^{c}	*Pseudomonas putida*
K1	1.19	31^{b}	*frederiksbergensis*
K2	1.53	48^{a}	*Burkholderia sabiae*
K3	1.23	35^{b}	*Pseudomonas mandelii*

Pot Experiment

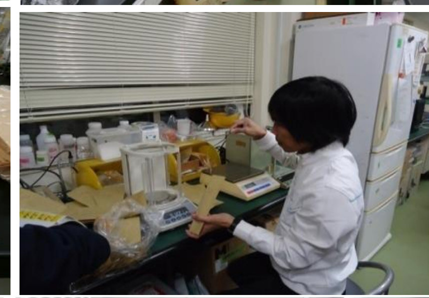
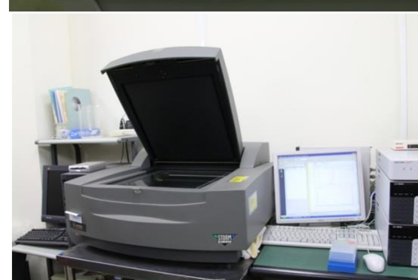
- Preparation of soil, sample gamma sterilization
- Initial soil analysis
- Potting
- KSB Inoculation
- Incubation and equilibration

Pot Experiment

- Germination test
- Planting
- Care and maintenance
- Harvesting
- Washing
- Weighing
- Grinding
- Radiometric analysis
- Plant autoradiography*
- Statistical analysis






Calculations

- Decay correction

$$A_o = A_i e^{\lambda t}$$

- Transfer factor in both shoots and roots

$$\mathrm{TF} = \frac{\mathrm{Cs\ in\ plants\ (Bq\ kg^{-1})}}{\mathrm{Cs\ in\ soil\ (Bq\ kg^{-1})}}$$

- Translocation ratio (TR)

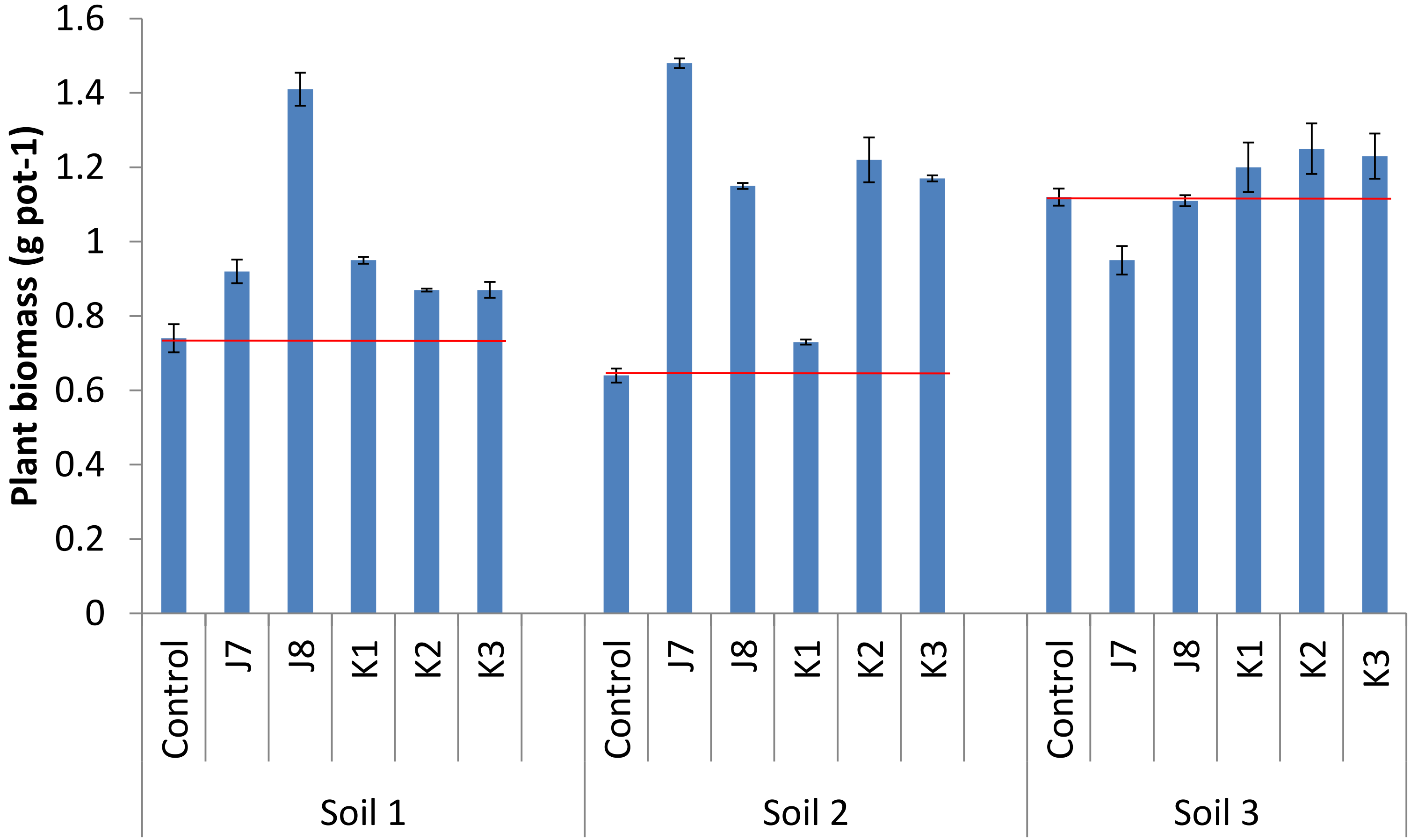
$$\mathrm{TR} = \frac{\mathrm{TF\ in\ plant\ shoots}}{\mathrm{TF\ in\ plant\ roots}}$$

Results

Effects of KSB isolates on shoot and root yield

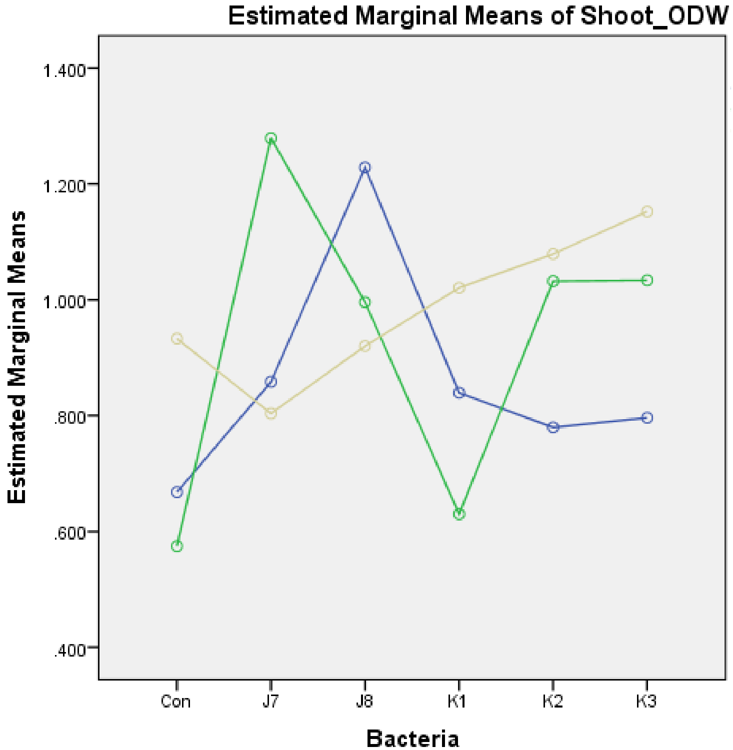
Soil	Isolates	Shoot (g)		Root (g)		Total Biomass (g)	Increase (%)
Soil 1	Control	0.67^{b}	± 0.05	0.07^{b}	± 0.01	0.74	-
	J7	0.86^{b}	± 0.06	0.06^{b}	± 0.01	0.92	25
	J8	1.23^{a}	± 0.07	0.18^{a}	± 0.01	1.41	90
	K1	0.84^{b}	± 0.13	$0.11a^{b}$	± 0.02	0.95	29
	K2	0.78^{b}	± 0.15	$0.09a^{b}$	± 0.01	0.87	18
	K3	0.80^{b}	± 0.09	0.07^{b}	± 0.02	0.87	18
Soil 2	Control	0.57^{c}	± 0.15	0.06^{b}	± 0.01	0.64	-
	J7	1.28^{a}	± 0.11	0.20^{a}	± 0.02	1.48	132
	J8	1.00^{b}	± 0.18	0.16^{ab}	± 0.04	1.15	81
	K1	0.63^{c}	± 0.07	0.10^{ab}	± 0.02	0.73	15
	K2	1.03^{b}	± 0.15	0.19^{a}	± 0.04	1.22	92
	K3	1.03^{b}	± 0.13	0.14^{ab}	± 0.03	1.17	83
Soil 3	Control	0.93^{ab}	± 0.06	0.19^{a}	± 0.02	1.12	
	J7	0.80^{b}	± 0.09	0.14^{a}	± 0.03	0.95	-5
	J8	0.92^{ab}	± 0.11	0.19^{a}	± 0.04	1.11	11
	K1	1.02^{ab}	± 0.12	0.18^{a}	± 0.03	1.20	20
	K2	1.08^{ab}	± 0.20	0.18^{a}	± 0.03	1.25	25
	K3	1.15^{a}	± 0.12	0.07^{b}	± 0.01	1.23	23

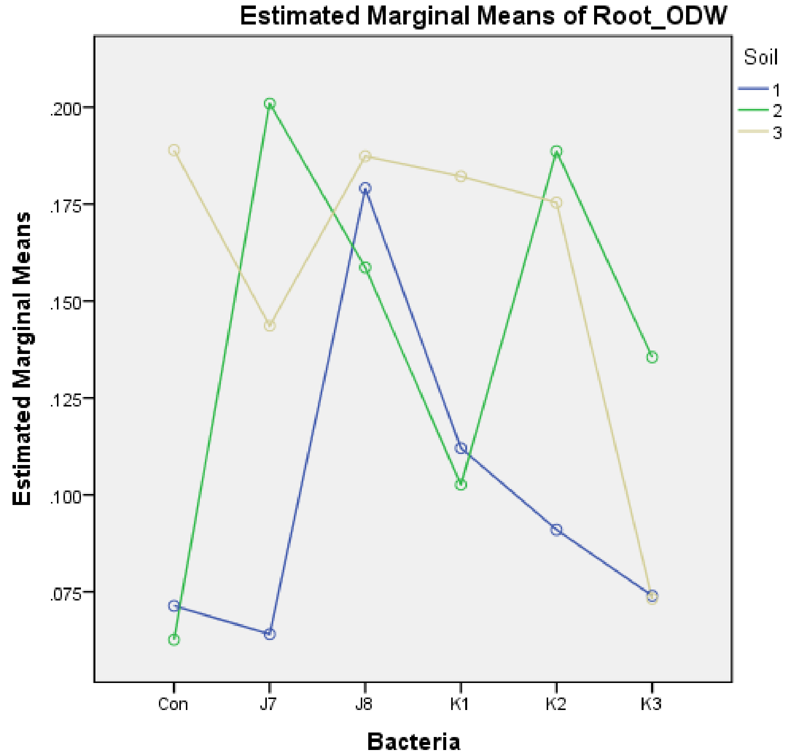
Results



Effects of KSB isolates on total biomass

Results



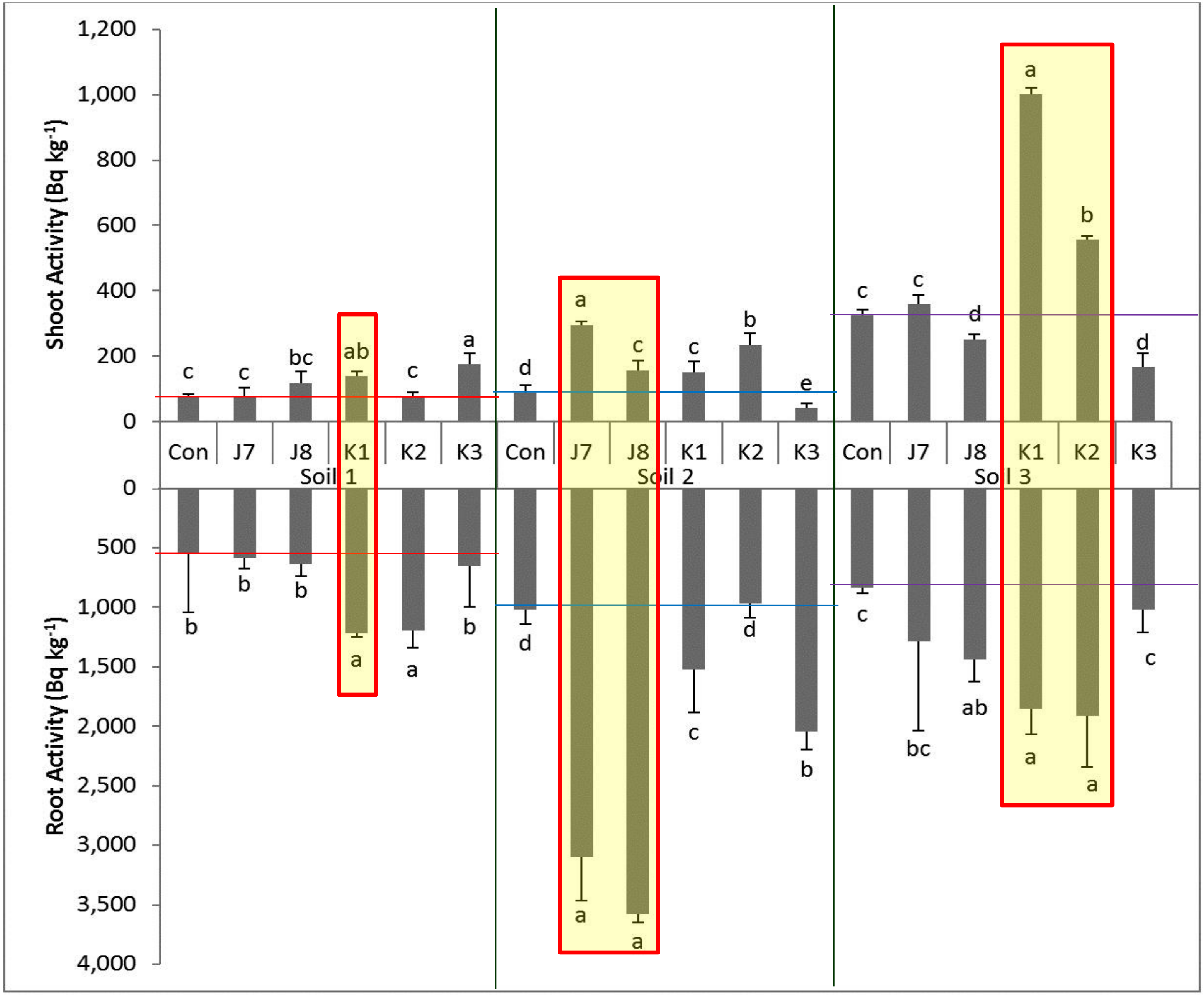


Interaction effects between soil and bacterial inoculants on shoot and root dry weight

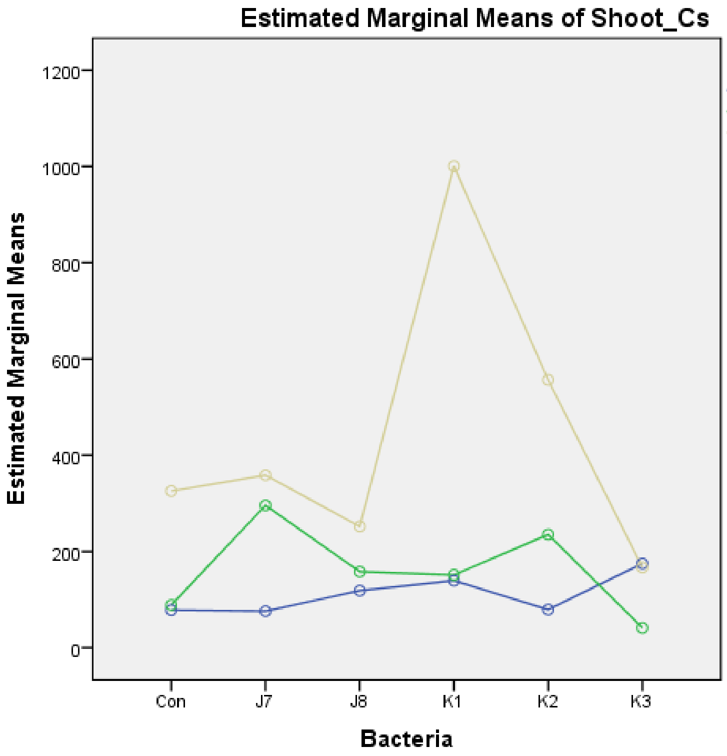
Results

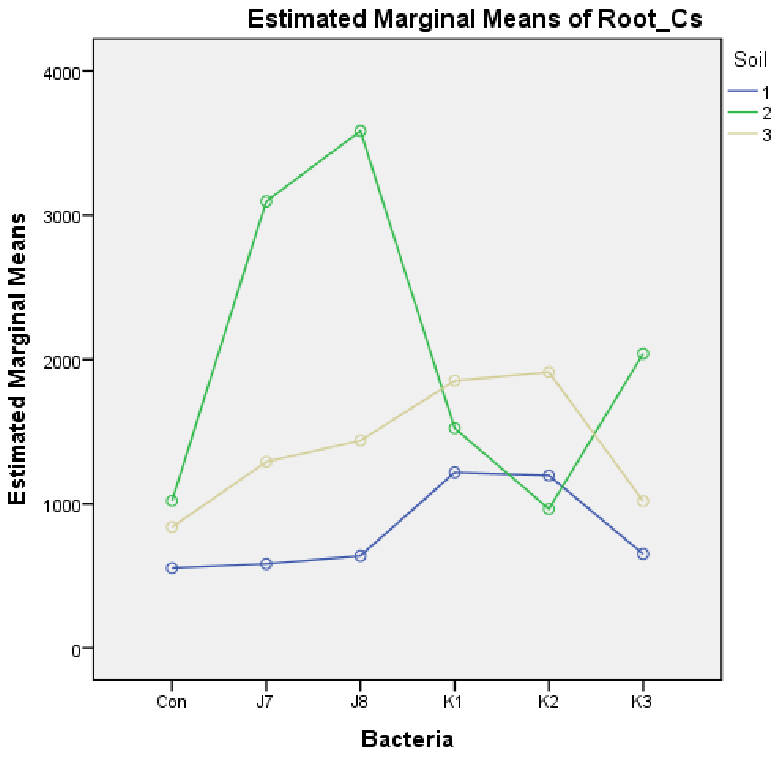
- Plant visual symptoms of possible radiotoxicity
- Cs interference in K metabolism in plants

Radiocesium accumulation in shoot and root

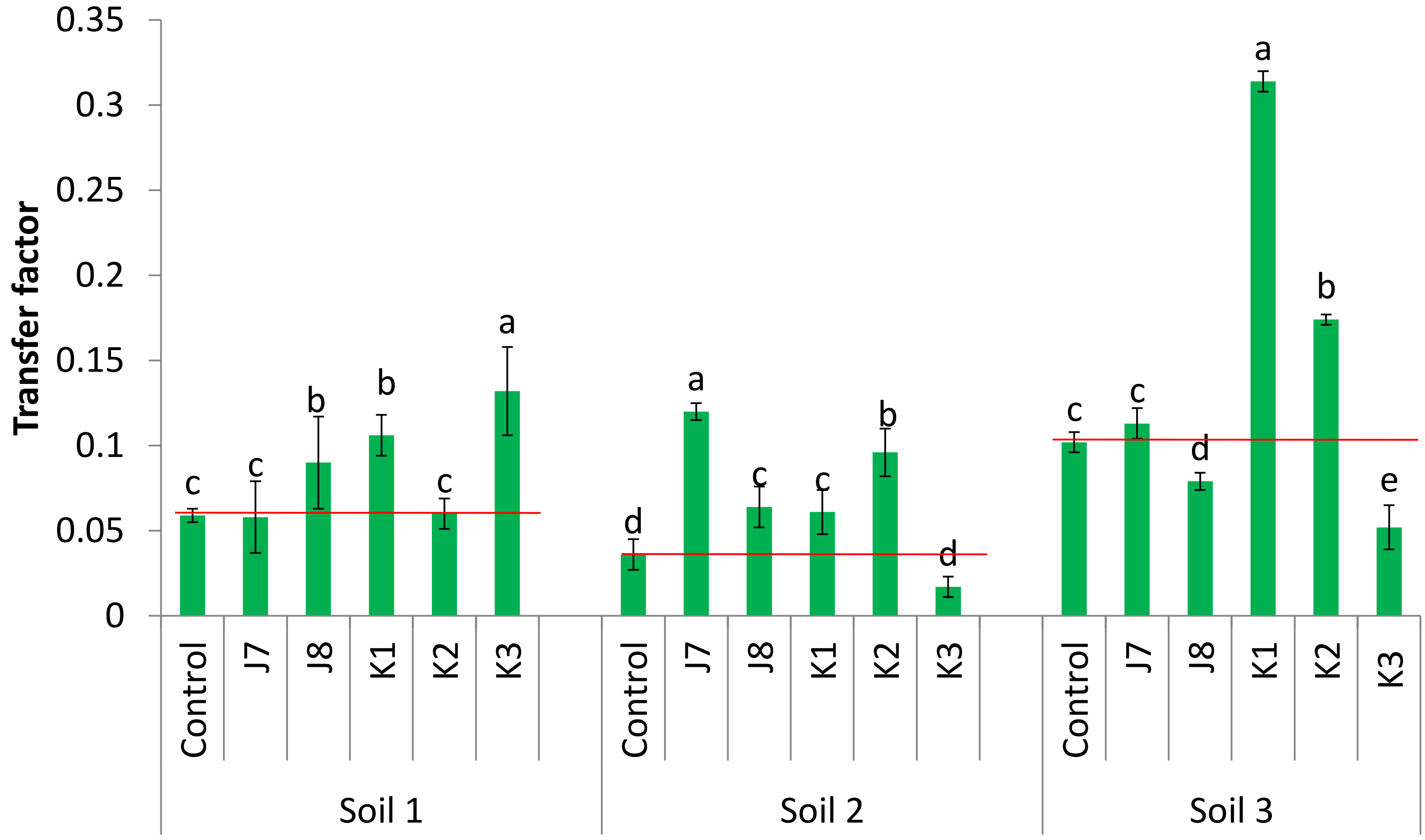


Results

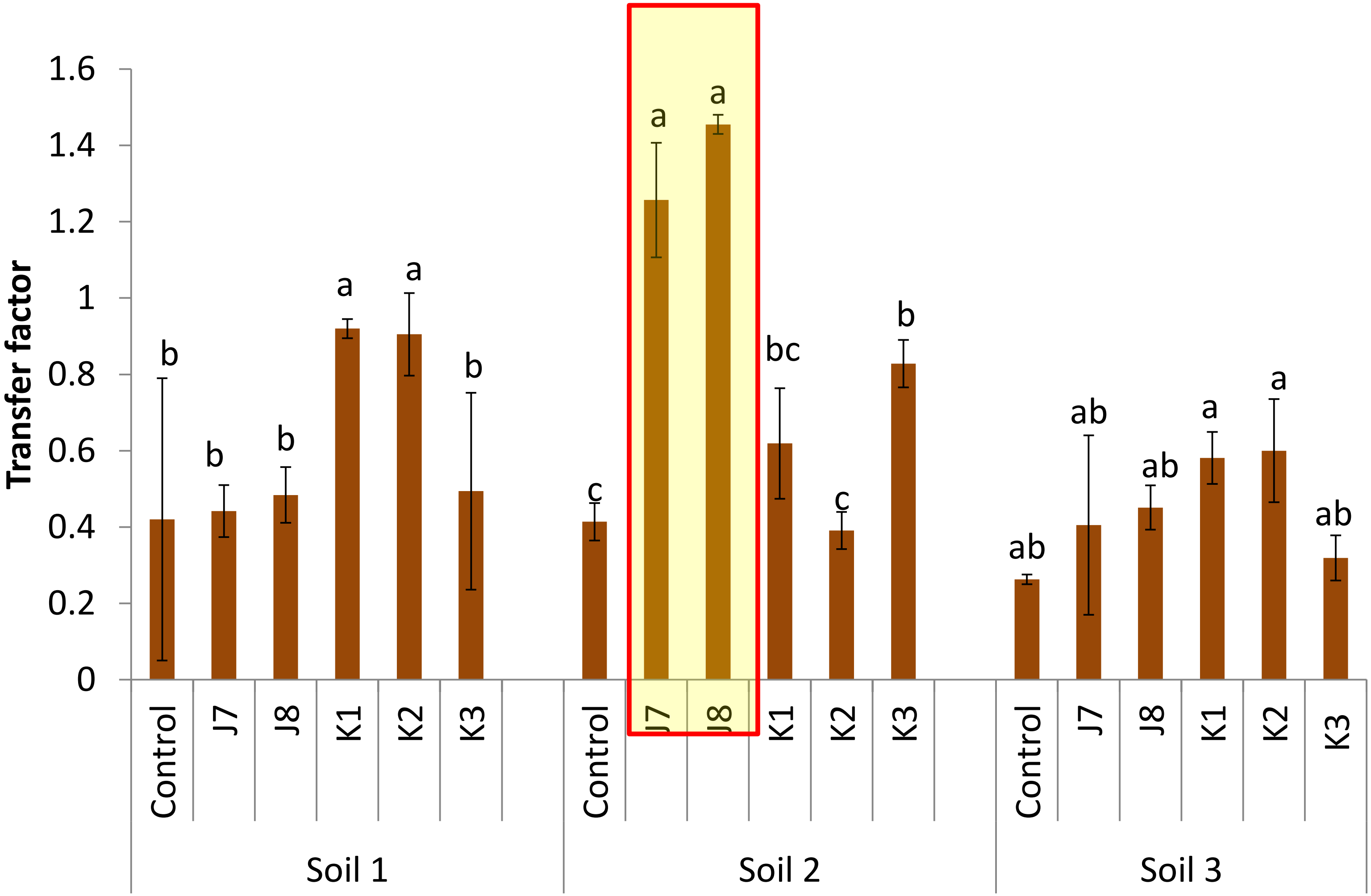




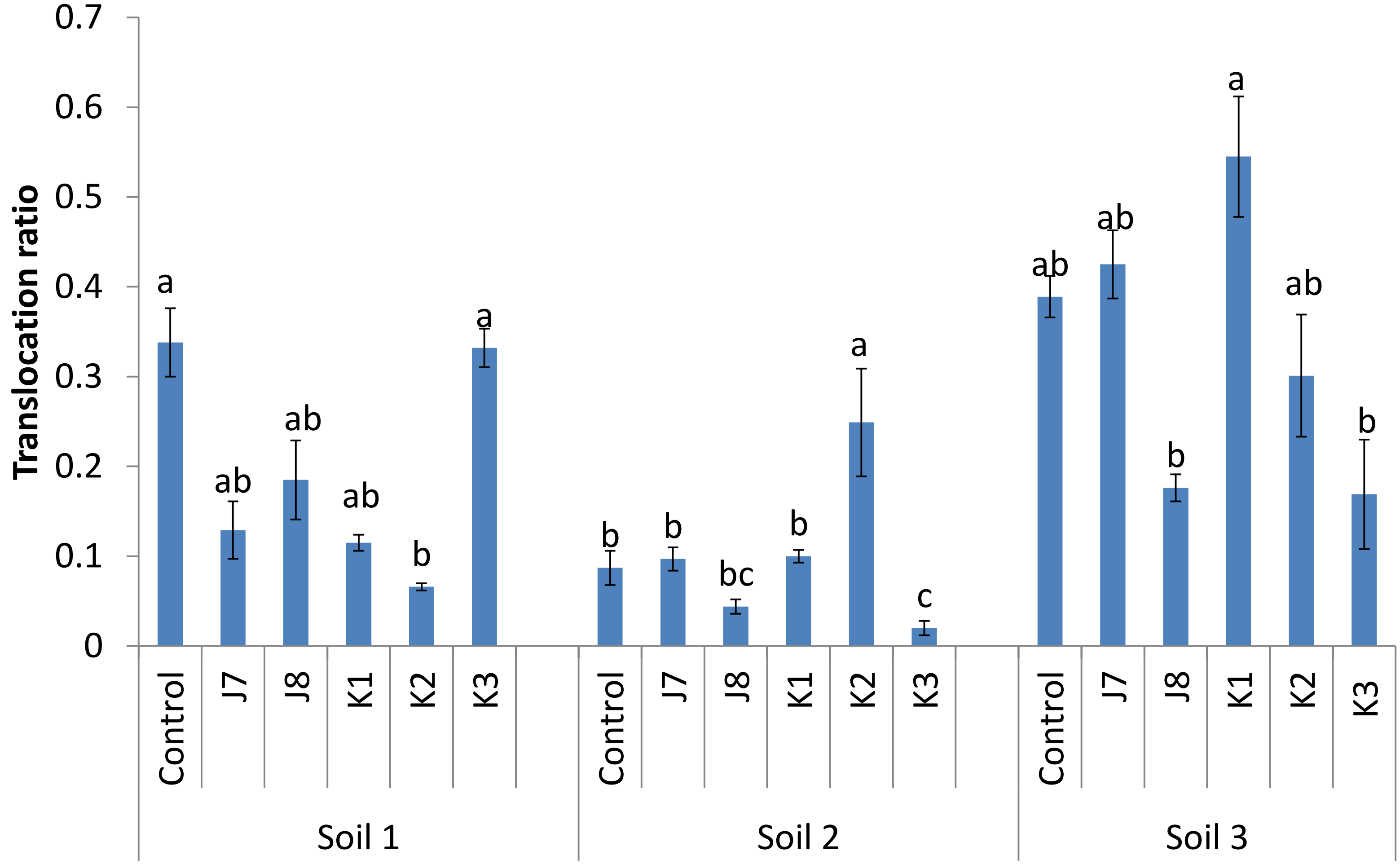
Interaction effects between soil and bacterial inoculants on root and shoot Cs



Transfer factors in shoot

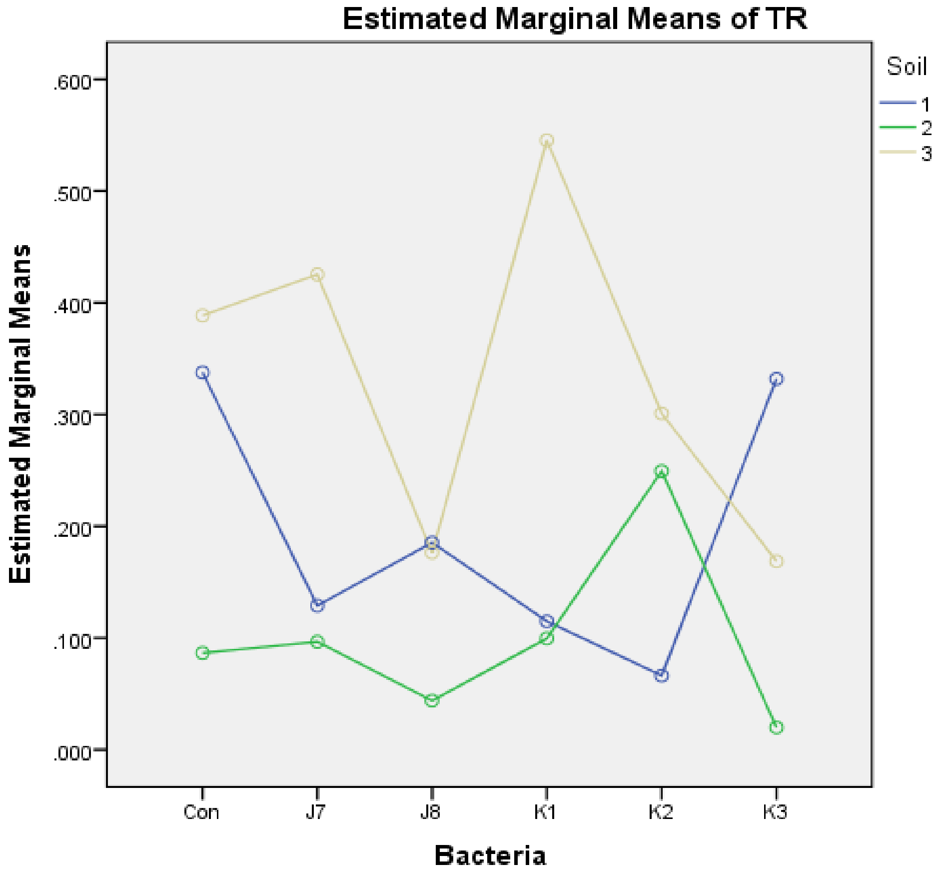


Transfer factors in root

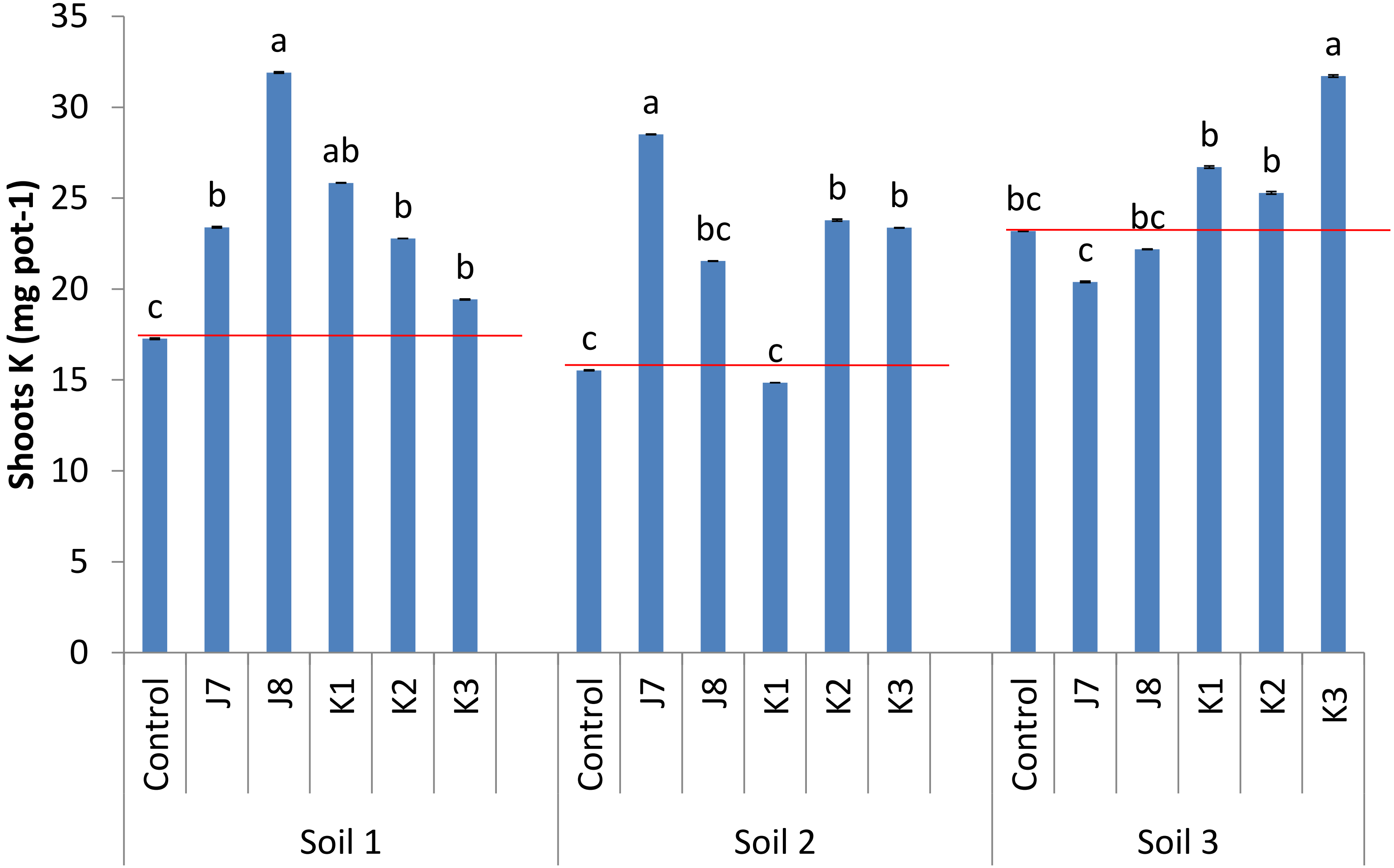


Translocation ratio

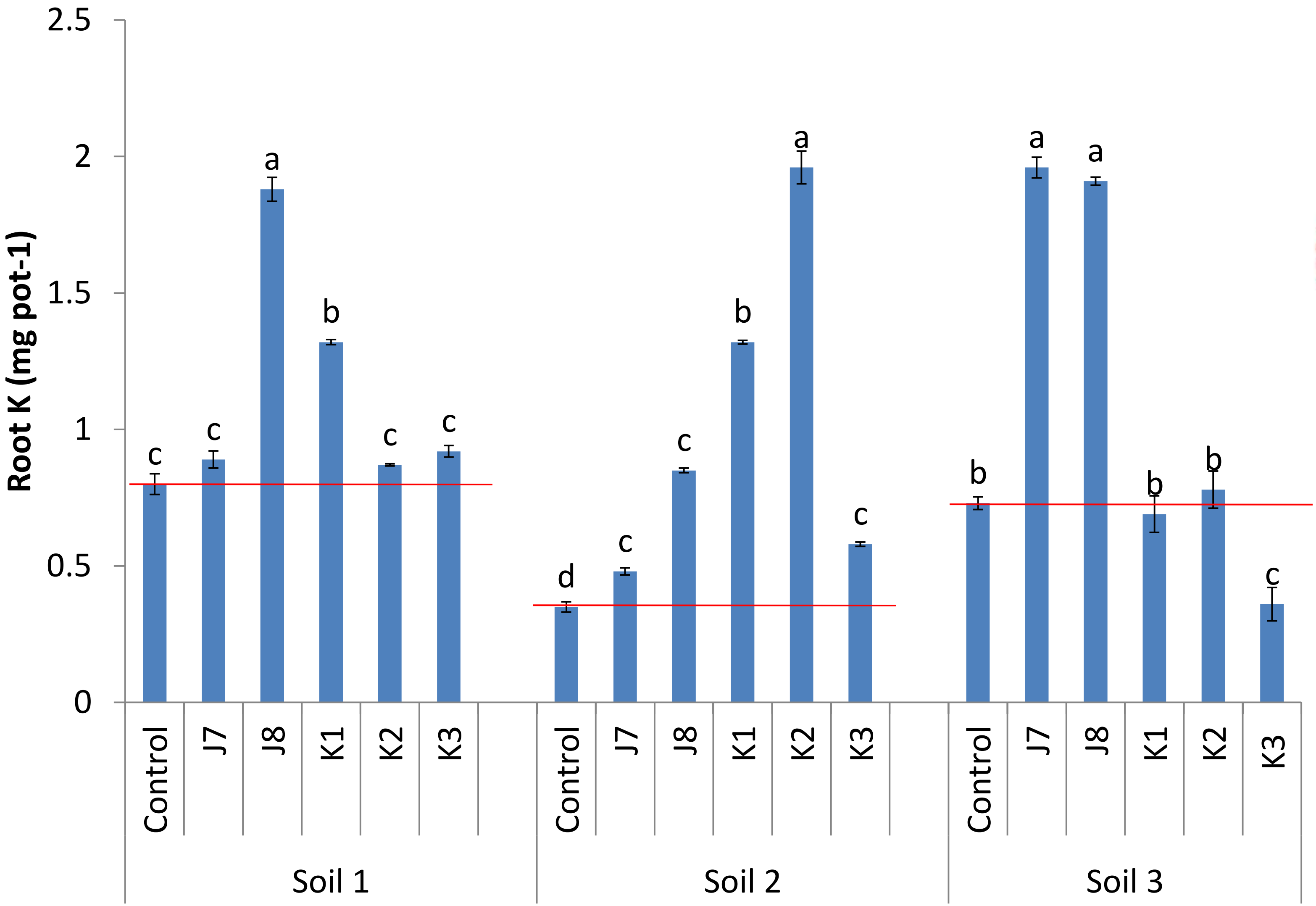
Results



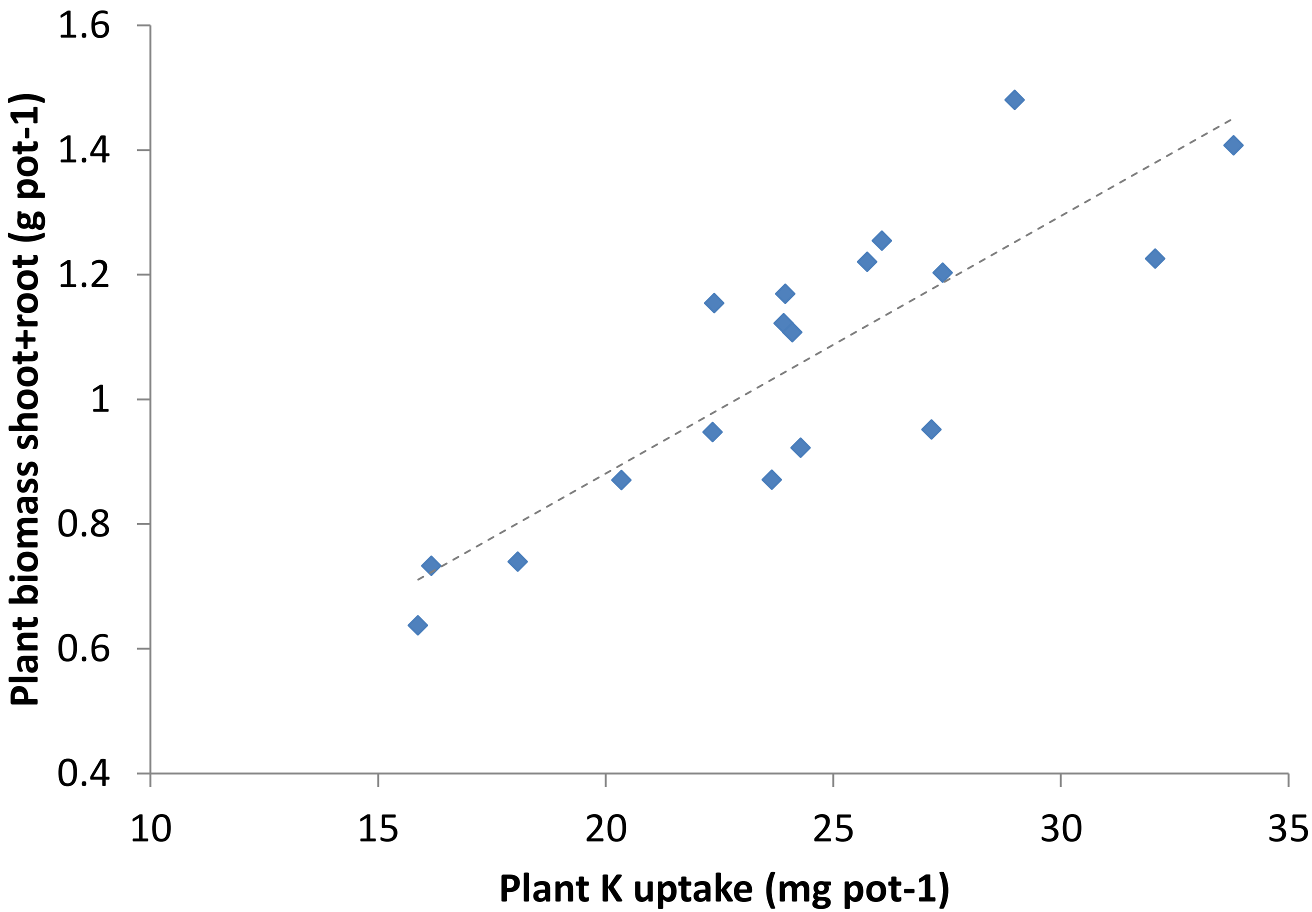
Interaction effects between soil and bacterial inoculants on Cs translocation ratio (TR)



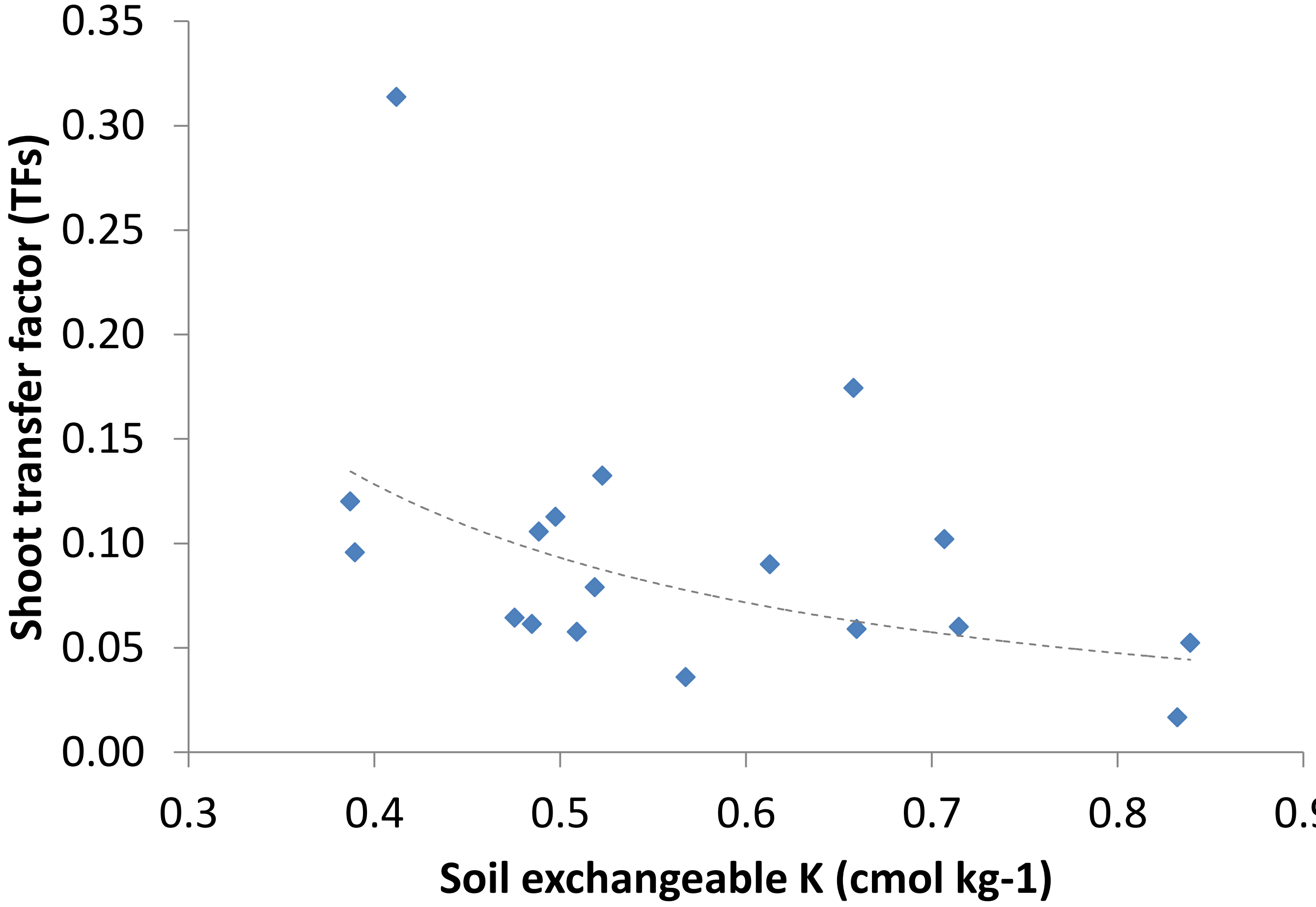
K plant uptake (shoots)



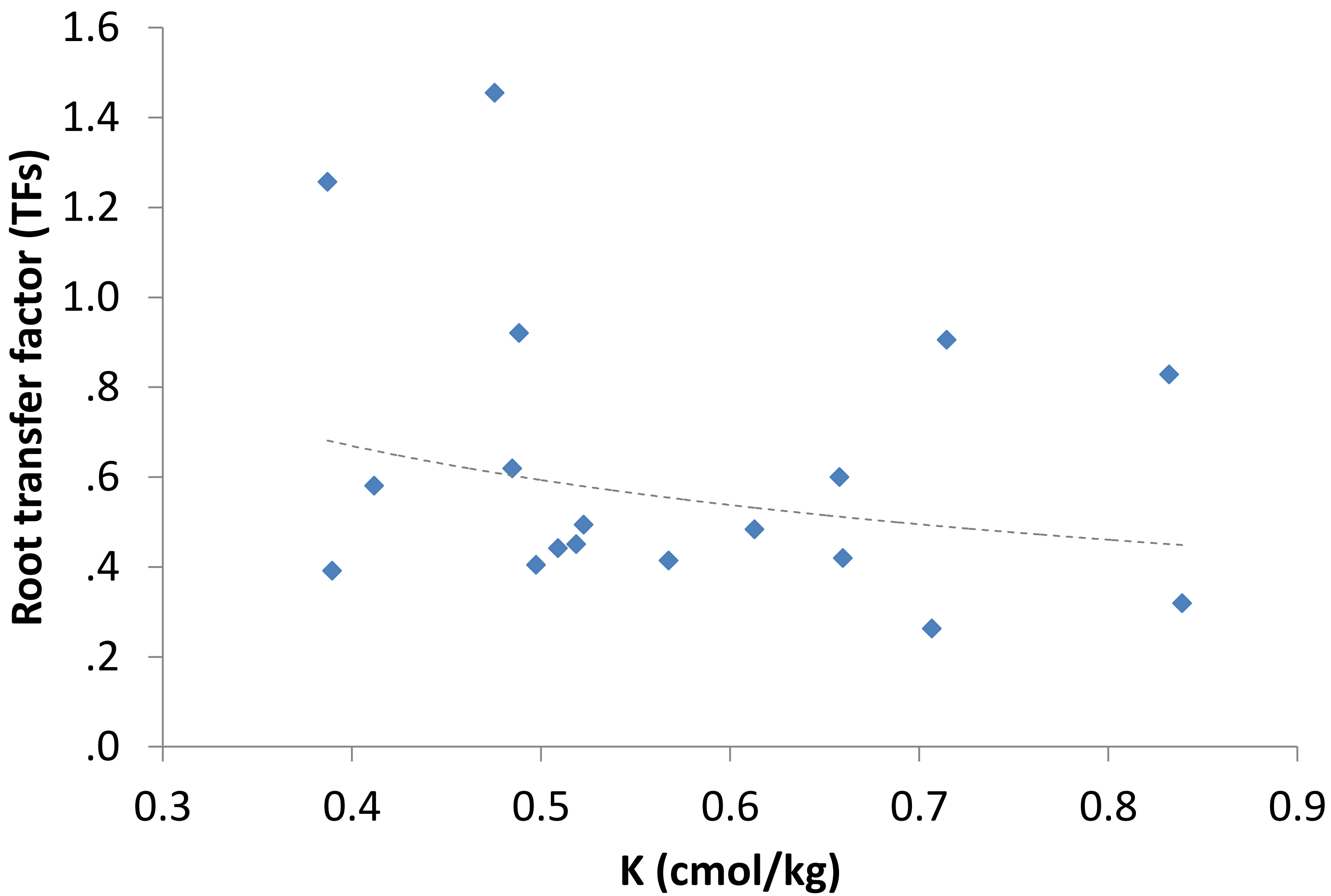
K plant uptake (roots)



Relationship between plant potassium uptake and total biomass



Relationship between soil exchangeable K and shoot transfer factor



Relationship between soil exchangeable K and root transfer factor

Conclusions

- KSB inoculation offers beneficial effects on plant growth and increased the overall plant biomass production
- KSB inoculation significantly increased the radiocesium accumulation, with much greater magnitude in roots than in shoots
- KSB inoculation may be essential in managing and manipulating radiocesium transfer from soils to plants
- workers interested in managing soil-to-plant transfer of radiocesium might consider the benefits of KSB on plant growth promotion and radiocesium accumulation

Recommendations

- supplementary research is needed on plants grown under actual field conditions in order to make reasonable predictions on the influence KSB inoculation on plant growth and radiocesium accumulation especially if the technology will be used to increase the efficiency of microbe-assisted phytoextraction.

Acknowledgements

- Ministry of Education, Culture, Sports, Science and Technology (MEXT)
- Nuclear Safety Regulation Authority (NSRA) of Japan
- Tokyo University of Agriculture and Technology (TUAT)