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Alteration in caesium behavior in rice caused by the potassium, phosphorous, and nitrogen deficiency

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Abstract

To provide a physiological basis for the impact of potassium (K) deficiency on the behaviors of caesium (Cs) in rice plant, the effects of phosphorus (P) and nitrogen (N) deficiencies were comparatively investigated. Then, 2 weeks of K-, P-, and N- deficiency all caused growth defect. Nevertheless, the increased Cs content in the shoot and the intensive transport of Cs to the young leaves were apparent only by the K starvation. It is suggested that the modification of Cs uptake and transport in rice plant is achieved by the distinctive physiological effect of K.

Keywords

potassium fertilization, *Oryza sativa*, *OsHAK1*, radiocaesium,

Introduction

On March 2011, a magnitude-9.0 earthquake hit the north-east region of Japan, followed by nuclear power plant explosions in Fukushima, which caused radiocaesium (^{134}Cs and

^{137}Cs) contamination to a variety of agricultural products. Above all, the contamination in rice has attracted considerable attention as it has been the staple food in Japan and the leading product of Fukushima. In aiming for the reduction of radiocaesium content in rice grain, one of the important subjects is to understand the mechanism of Cs accumulation in rice grain. The previous study carried out in an experimental field replicating the typical paddy field showed that caesium (Cs) absorbed by rice tended to accumulate at higher concentration in old leaves than in younger leaves and panicles [1]. In contrast, it was found that, in Fukushima prefecture, there were rice plants showing higher radiocaesium levels in the younger leaves than that in the older leaves, and producing highly contaminated brown rice grains (approximately 500 Bq kg^{-1}) [2]. We previously reported that the potassium (K) deficiency contributed the enhanced radiocaesium translocation from old leaves to younger organs, which eventually lead to higher radiocaesium accumulation in younger tissues including brown rice [3].

On the other hand, the influence of the depletion of other nutrients on the Cs distribution pattern remains elusive. Nitrate (N) and phosphate (P) are the macro-nutrients whose shortages due to the outflow from paddy fields during irrigation and planting are of major concern in agriculture [4]. Therefore, N and P fertilization has been the important determinants of yield, and, in this regard, the better irrigation systems have been devised and evaluated based on the behavior of N and P [5]. In this study, the effect of N and P deficiency on Cs accumulation in rice shoots was studied by the ^{137}Cs tracer experiment with hydroponically grown rice, and compared with the effect of K deficiency.

Experimental

Plant growth conditions and ^{137}Cs treatment

Rice (*Oryza sativa* L. ‘Nipponbare’) seeds were germinated in pure water for 3 days and transferred to 0.5 mM $CaCl_2$ solution. After 2 days, the seedlings were supplied with the modified half-strength Kimura B nutrient solution which contained 270 μM K^+, 370 μM NH_4^+, 460 μM NO^{3-}, and 92 μM PO_4^{3-} (pH 5.6) [6] for 2 days. Then, the rice seedlings were transferred to 300 mL plastic pots with seven different culture solutions; control (half-strength Kimura B), K-free, P-free, N-free solutions, and the solutions with either K, P, or N concentration of one-tenth of the control. Half-strength Kimura B solution was modified to reduce K or N or P by replacing KH_2PO_4, K_2SO_4, and KNO_3 with sodium salts for K-deficient solutions, $(NH_4)_2SO_4$, KNO_3 and $Ca(NO_3)_2$ with K_2SO_4 and $CaCl_2$ for N-deficient solutions, and KH_2PO_4 with KCl salts for P-deficient solutions. All the solutions contained 0.1 μM Cs and supplied with non-carrier-added ^{137}Cs (Eckert & Ziegler CNL Scientific Resources) at 5.9 kBq L^{-1} as a radiotracer. After transferring, the plants were grown for another 2 weeks at 30°C with a 12:12 h light:dark photoperiod. The culture solution was changed twice every week.

Sampling and ^{137}Cs measurement

Two weeks after the ^{137}Cs treatment, 3 seedlings for each treatment were harvested and separated into each leaf and the root. The weight of each tissue was measured. The 2 youngest leaves were collectively named as "New leaves" and the other shoot part were designed as "Old leaves". The radioactivity of each tissue was measured using a well-type NaI(Tl) scintillation counter (ARC-300, Aloka, Tokyo, Japan) for 5 minutes to obtain the net count more than 1,000. The hight of the samples charged to the disposable sample-tube was less than 1 cm. The concentration of Cs in each tissue was calculated based on the ^{137}Cs activity and the biomass.

OsHAK1 expression analysis in roots

The roots of one-week-old rice plants (*Oryza sativa* L. 'Nipponbare') grown under K-free and control nutrient solutions were sampled and immediately frozen in liquid nitrogen. Total RNA was extracted from each root using the Trisure (BIOLINE) reagent. Reverse transcription was performed with SuperScript III (Invitrogen) according to the manufacturer's protocol. Real-time quantitative RT-PCR analysis was performed according to the instruction of Fast SYBR Green (Applied Biosystems). The OsHAK1 (accession; AJ427970) cDNA was amplified using a forward primer 5'-ATCTAATGGGAGAGACCGAG-3' and a reverse primer 5'-AACTCATAGGTCATGCCAAC-3'. The copy number of OsHAK1 cDNA was determined by being normalized with the transcript of UBC gene [7] as an internal control by the following calculation.

$$\text{Relative HAK1 expression} = 2^{\text{Ct UBC} - \text{Ct OsHAK1}}$$

The sequence of UBC primers was 5'-ATTTGGGTCGCGGTTCTTG-3' (forward) and 5'-TGCCTTGACATTCTCGATGGT-3' (reverse).

Results and discussion

. We previously reported that, in addition to the increased Cs uptake activity, the modified Cs distribution pattern within the rice plant in response to the environmental K condition was found [2]. Here, the possibility that the distribution of Cs within the rice plant could be modified specifically by the K deficiency treatment was examined. The total biomass was found to be decreased as the nutrient deficiency was stricter (Fig. 1a). In particular, the biomass of rice samples grown in the K-free, one-tenth-N, and N-free solution was reduced by less than half of that in the control sample (Fig. 1a), and the growth retardancy was apparant in those samples. The Cs uptake amount in the K-starved rice plant was increased up to 4-fold of the control condition and the Cs concecntration was increased accordingly (Fig. 1b), which was probably due to the enhanced root uptake of Cs as reported previously [2, 8]. In addition, in response to the K starvation, the Cs distribution was altered and the concentrations in the new leaves exceeded those in the old leaves (Fig. 1b). In contrast, the Cs concentration in N deficient and P deficient rice plant was decreased especially in the root (Fig. 1b). In addition, both P deficiency and N deficiency were shown to have a little effect on the Cs allocation between the new leaves and old leaves (Fig. 1b). These results suggest that the modified Cs allocation between the old tissues and young tissues could be characteristically caused by the low K condition in the growth medium, but not by the growth defect as a consequence of poor nutrient condition. To further link the modified Cs allocation to the K deficiency response, we checked the expression status of *OsHAK1*, frequently used as the marker for K deficiency [9]. Then, as expected, upregulation of *OsHAK1* expression in the root of the

K starved rice seedlings was found (Fig. 2). It strongly indicated that the rice plant grown in the K-free solution in our experimental procedure was responding to the low K condition at the transcription level, which could further adopt some physiological activities, including ion transport, inside the plant.

In the paddy-field, it is noteworthy that the fertilization supply also affects on the Cs contamination in the context of soil physics other than of plant physiology. For example, the increased Cs concentrations both in straw and brown rice were observed when N fertilization was provided, probably because Cs fixed with soil was exchanged with ammonium ion, increasing the available Cs in the rhizosphere [10]. Hence, it is important to understand the influence of the fertilization supply on both plant physiology and soil physics to determine the effective agricultural strategy for reducing Cs contamination to rice.

Figure captions

Fig. 1 The effect of nutrient deficiency on the Cs behavior in rice. Samples were grown either in the full nutrient solution (control), K-deficient (one-tenth-K and K-free) solutions, P-deficient (one-tenth-P and P-free) solutions, or N-deficient (one-tenth-N and N-free) solutions for 2 weeks. (a) Biomass (mg) in fresh weight, and (b) the Cs concentration in the root, old leaves, and new leaves (pmol/mg). Means with the standard deviations were presented (n=3, biological replication)

Fig. 2 Expression of *OsHAK1* gene in the root of the control and K deficient (0mM K) rice. Means with standard deviations (n=3) were presented

Conclusions

To reduce the radiocaesium contaminatino in rice, maintaing the adequate level of K in the soil should be effective in consideration of the physiological relationship beween Cs and K. By combining these physiological aspects and other environmental aspects such as soil science, the practical management system for farm work could be achieved.

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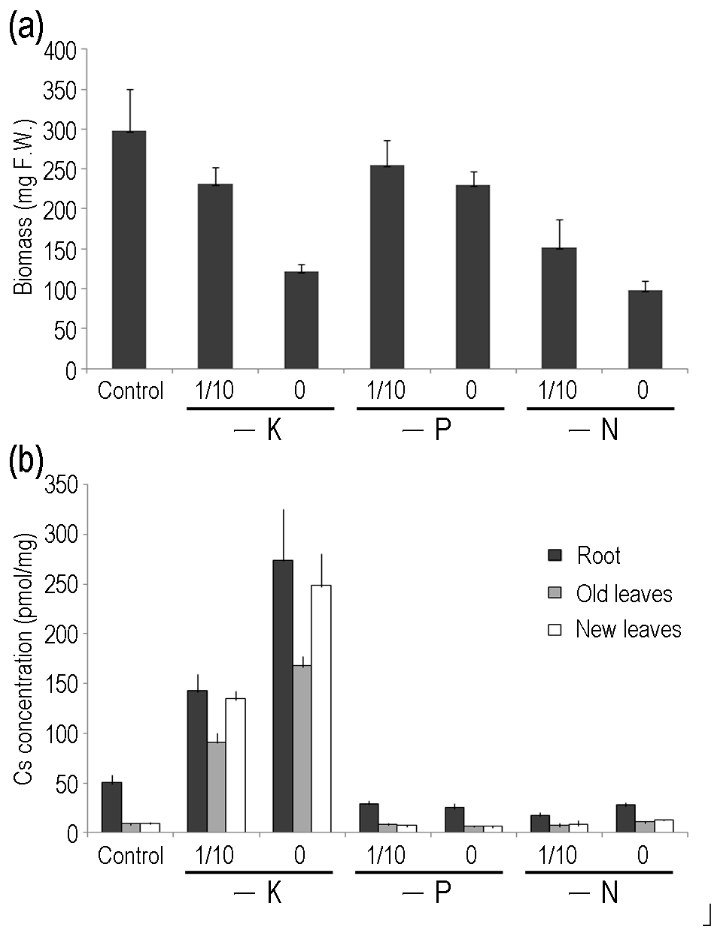
(a)
Biomass (mg F.W.)
400
350
300
250
200
150
100
50
0
Control
1/10
0
1/10
0
1/10
0
— K
— P
— N
(b)
Cs concentration (pmol/mg)
350
300
250
200
150
100
50
0
Root
Old leaves
New leaves
Control
1/10
0
1/10
0
1/10
0
— K
— P
— N

Fig. 1

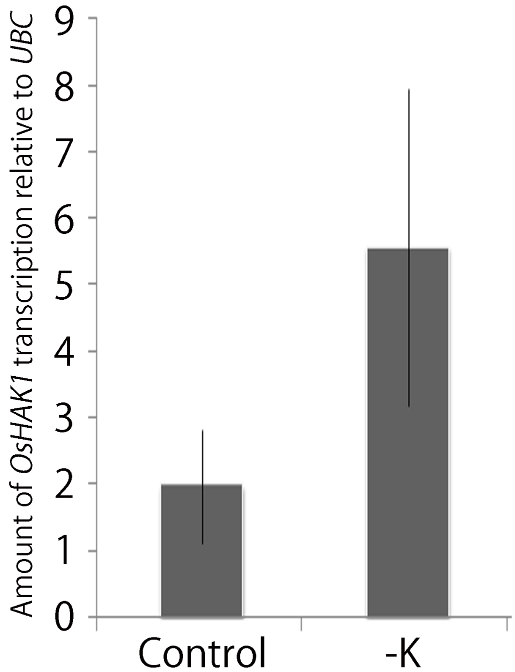
Amount of OsHAK1 transcription relative to UBC
0
1
2
3
4
5
6
7
8
9
Control
-K

Fig.2