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SHORT COMMUNICATION



## Internal Cs<sup>+</sup> inhibits root elongation in rice

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### ABSTRACT

The root system anchors the plant to the soil and contributes to plant autotrophy by taking up nutrients and water. In relation with this nutritional function, root development is largely impacted by availability of nutrients and water. Due to human activity, plants, in particular crops, can also be exposed to pollutants which can be absorbed and incorporated into the food chain. Cesium in soils is present at non-toxic concentrations for the plant (micromolar or less), even in soils highly polluted with radioactive cesium due to nuclear accidents. Here, we report on the morphological response of rice roots to Cs<sup>+</sup> at micromolar concentrations. It is shown that Cs<sup>+</sup> reduces root elongation without affecting root dry weight. Noteworthy, inactivation of the Cs<sup>+</sup>-permeable K<sup>+</sup> transporter OshAK1 prevents such effect of Cs<sup>+</sup>, suggesting that internal Cs<sup>+</sup> triggers the modification of the root system.

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Roots are the underground interlocutor of the environment. Not only able to take up nutrients and water from the soil, they also sense and respond to fluctuations in their availability.<sup>1-4</sup> In the recent decades, due to climate change, environmental conditions have become harsher for plants with an increase in time and intensity of abiotic stress periods (drought, salinity, heat, waterlogging, etc.).<sup>5</sup> Moreover, human activity is leading to the occurrence of pollutants in both natural ecosystems and agricultural fields. Thus, plants are likely to be affected by complex interactions emerging from this changing environment.

In recent years, radiocesium has gained much attention due to the Fukushima accident in 2011 which has given rise to the release of large amounts of radiocesium to the environment. This sort of disaster has a big impact in terms of time and surface, since radioactive cesium (Cs) isotopes, <sup>134</sup>Cs and <sup>137</sup>Cs have relatively long half-lives: 2-year and 30-year half-life, respectively, and contamination can be spread to vast regions by air and water.<sup>6</sup> Agriculture is particularly affected by radiocesium since crops take up Cs<sup>+</sup> from contaminated soils and this constitutes the entry of this pollutant in the trophic chain. Cs<sup>+</sup> can be toxic for plants at mM concentrations but radiocesium is present at much lower concentrations ( $\mu$ M or less) which does not lead to visible toxicity symptoms.<sup>7</sup> Rice is the major crop affected by radiocesium in Japan and extensive efforts are being made to minimize its accumulation in aerial parts.<sup>8</sup>

In order to gain insights into the effects of Cs<sup>+</sup> in rice plants at low concentrations, we performed a series of experiments in hydroponics conditions using <sup>133</sup>Cs<sup>+</sup>, the

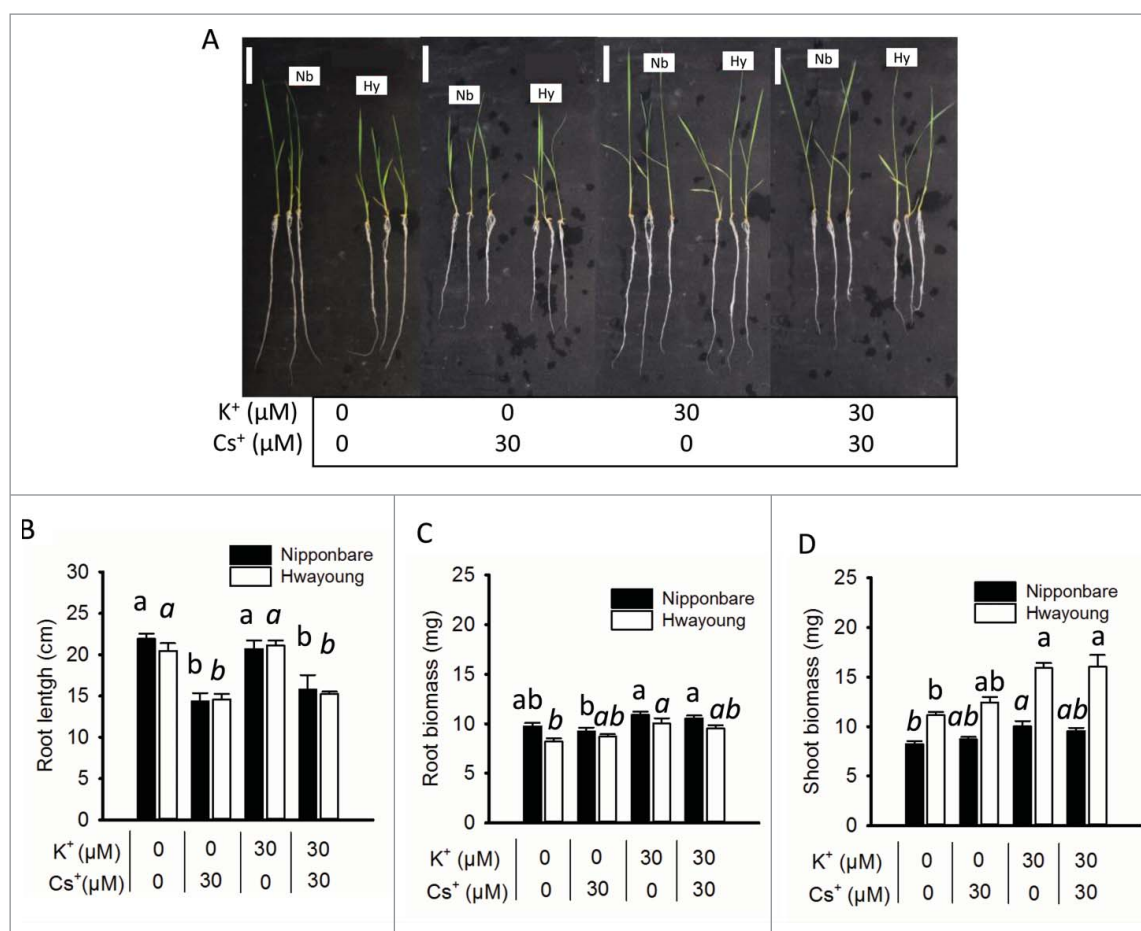
non-radioactive isotope. Since K<sup>+</sup> and Cs<sup>+</sup> compete to enter into rice roots,<sup>9-11</sup> we measured the growth of two rice cultivars, Nipponbare and Hwayoung, under a combination of Cs<sup>+</sup> and K<sup>+</sup> micromolar concentration supplies: 0K-0Cs, 0K-30Cs, 30K-0Cs and 30K-30Cs (Fig. 1). The most remarkable effect was that both Nipponbare and Hwayoung plants treated with Cs<sup>+</sup> (0K-30Cs and 30K-30Cs) had shorter root systems, by about 25% (Fig. 1A & B). It is worth to note that this effect was independent of the K<sup>+</sup> supply and it did not affect total root biomass (Fig. 1C). Neither shoot biomass reduction (Fig. 1D) nor leaf chlorosis (Fig. 1A) were observed in the 2 Cs<sup>+</sup> treatments, which suggested that the Cs<sup>+</sup> concentration employed (30  $\mu$ M) was not toxic. It should be noted that cesium has also been shown to reduce root length in Arabidopsis.<sup>12</sup> However, this response was observed at toxic concentrations (>100  $\mu$ M), which are expected to produce, additionally, lower root and shoot dry weight.

K<sup>+</sup> contents of root and shoot tissues of rice cv Nipponbare grown in the above conditions were recently reported by Nieves-Cordones et al.<sup>11</sup> Interestingly, the presence of 30  $\mu$ M Cs<sup>+</sup> in the growth medium had no impact on the plant tissue K<sup>+</sup> content. Thus, the inhibition of root elongation in the presence of 30  $\mu$ M Cs<sup>+</sup> in the growth medium was not due to K<sup>+</sup> deficiency in the plant tissues. Our results rather suggest that Cs<sup>+</sup> is sensed by rice roots and triggers change in root morphology. We conducted a second series of experiments aimed at investigating whether Cs<sup>+</sup> perception is internal or external. Nieves-Cordones et al. recently reported the very low capacity to accumulate Cs<sup>+</sup> in rice plants produced with CRISPR-Cas technology which lacked the Cs<sup>+</sup>-permeable K<sup>+</sup> transporter

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**Figure 1.** Cesium ions inhibit root elongation. (A) Photograph of rice plants, from Nipponbare and Hwayoung cultivars, grown for 14 d in hydroponics under a combination of Cs<sup>+</sup> and K<sup>+</sup> micromolar supplies (0K-0Cs, 0K-30Cs, 30K-0Cs and 30K-30Cs). Plant growth conditions are described in<sup>11</sup>. Briefly, plants were germinated in wetted filter paper and then grown hydroponically on 1 mM CaSO<sub>4</sub>, 2 mM MES pH 6.0 at 28°C day/25°C night 80% humidity during 14 d. After 7 d, 30 μM CsCl and/or 30 μM KCl were added to the growth solution as indicated. Scale bar = 5 cm. (B) to (D) Root length (B), root (C) and shoot (D) biomass of rice plants at the end of the experiment. Root length depicts the maximum length of the root system for each plant. Values correspond to means ± SE, n = 3. Letters denote different groups according to Tukey's test (*P* < 0.05). Tukey's test was performed separately for each cultivar (letters corresponding to Hwayoung plants are in italics).

OshAK1<sup>11</sup>. These *oshak1* plants are impaired in root Cs<sup>+</sup> uptake and they show a very low internal Cs<sup>+</sup> content when grown at external micromolar Cs<sup>+</sup> concentrations.<sup>11</sup> Therefore, they could serve as an interesting tool to identify the origin of the Cs<sup>+</sup> signal. For example, if Cs<sup>+</sup> perception is external, this mutant should behave like WT plants when grown in the presence of Cs<sup>+</sup>. Thus, we checked Cs<sup>+</sup>-mediated root elongation inhibition in *oshak1* plants. Rice roots of WT-c (control with intact *OshAK1* gene) and *oshak1* (containing frameshift mutations in the *OshAK1* gene<sup>11</sup>) were grown in 0K-0Cs and 0K-30Cs and length of individual roots (primary and seminal) was measured (Fig. 2). In WT-c roots, a shift towards shorter roots in root length classes was observed when plants were grown in the presence of Cs<sup>+</sup> (Fig. 2A). This result is consistent with the decrease in primary root length previously described (Fig. 1B). Such shift in root length classes was not any longer observed in *oshak1* roots (Fig. 2B). It is noteworthy that, again, root biomass was not significantly affected by the Cs<sup>+</sup> treatment in WT-c and in *oshak1* plants (Fig. 2C). The fact that roots are shorter in WT plants upon Cs<sup>+</sup> treatment but keep the same root biomass is currently under further investigation. Finally, we measured root Cs<sup>+</sup> content in WT-c and in *oshak1*

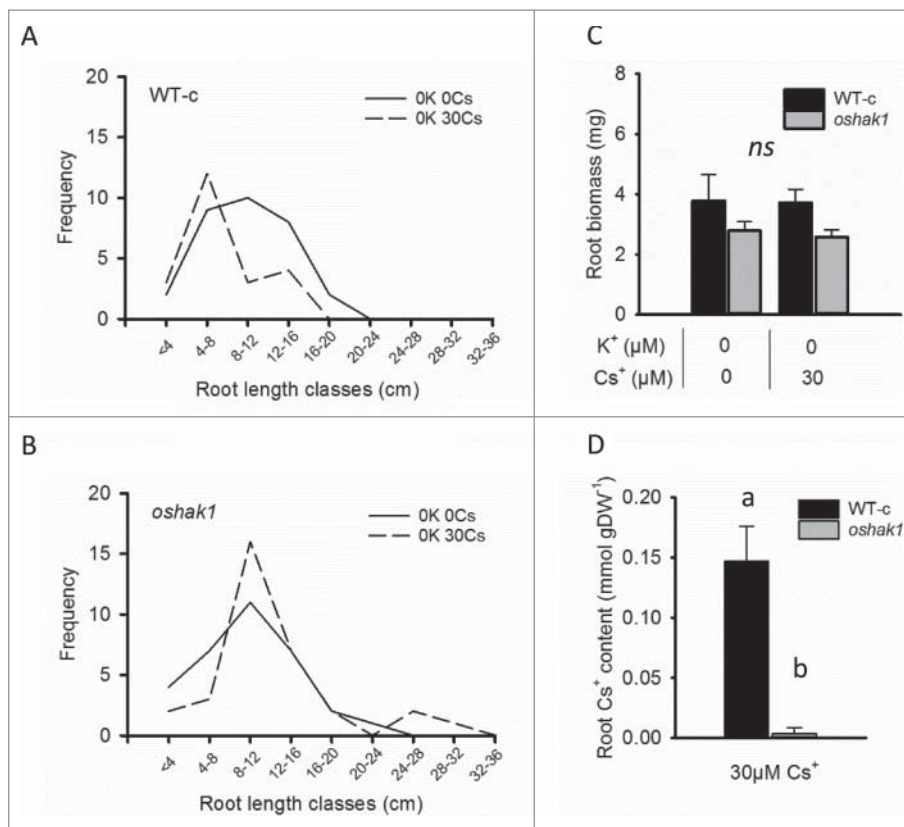
plants and found that it was 44 times lower in mutant plants than in WT-c plants (Fig. 2D). Thus, Cs<sup>+</sup>-mediated root elongation inhibition is not observed in absence of significant root Cs<sup>+</sup> accumulation, which suggests that internal rather than external Cs<sup>+</sup> is mediating this response. At this stage, it cannot be ruled out that Cs<sup>+</sup> transport carried out by OshAK1 is part of the sensing mechanism and is therefore required to exhibit the aforementioned root phenotype. Nevertheless, the Cs<sup>+</sup>-induced root elongation inhibition may have implications for rice growing in Cs<sup>+</sup>-rich soils. Insights into this issue will require further research.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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**Figure 2.** Cs<sup>+</sup> dependent inhibition of root elongation requires Cs<sup>+</sup> uptake and accumulation in root tissues. (A) and (B) root length classes of WT-c (A) and *oshak1* (B) plants grown in the OK-0Cs and OK-30Cs treatments. *oshak1* plants were generated with the CRISPR-Cas system as described in.<sup>11</sup> A single guide RNA (sgRNA) complementary to a 20 bp sequence at exon 2 of the *OsHAK1* locus was designed and coexpressed with Cas9 in seed embryo-derived rice calli. Gene-edited and control plants were selected after plant regeneration. *oshak1* T1 plants contain -2 bp, -4 bp frameshift mutations at positions +2075 bp and, respectively, +2073 pb in the *OsHAK1* gene. WT-c control plants have no mutation in *OsHAK1*. Root length of every seminal and primary root was measured with ImageJ and values were sorted into classes. (C) Root biomass of WT-c and *oshak1* plants grown in the OK-0Cs and OK-30Cs treatments. (D) Root Cs<sup>+</sup> content of Cs<sup>+</sup>-treated plants. Values correspond to means  $\pm$  SE, n = 5-7. Letters denote different groups according to Tukey's test ( $P < 0.05$ ). ns stands for non-significant ( $P > 0.05$ ) difference between plants from the same genotype grown in different ionic conditions.

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