

## EFFECTS OF KINETIN AND GIBBERELIC ACID ON GROWTH AND ION TRANSPORT OF RICE UNDER CADMIUM STRESS

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### Abstract

An experiment was conducted to analyze the effects of kinetin and gibberellic acid on growth, and ion transport of rice (*Oryza sativa* var. BRRI-80) under cadmium stress. Exogenous application of both kinetin (2  $\mu\text{M}$ ) and gibberellic acid (1  $\mu\text{M}$ ) alleviated the negative effects of cadmium (Cd) on growth parameters by enhancing the length of both root and shoot, and biomass of Cd stressed rice plants grown on sand culture. Kinetin and GA<sub>3</sub> caused decrease in Na<sup>+</sup> accumulation while increasing K<sup>+</sup> accumulation in both root and shoot. Accumulation of NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> were improved under cadmium stress by kinetin and GA<sub>3</sub> in cadmium stressed rice plants. In addition to this, kinetin and GA<sub>3</sub> reduced Cd accumulation in the root and shoot of cadmium stressed rice plants. Both kinetin and gibberellic acid were found effective in improving relative water contents in shoots of cadmium stressed rice plants.

### Introduction

Heavy metal pollution of soil has become one of the most leading environmental problems in the world (Ali *et al.* 2014). Cadmium is one of the most toxic heavy metals at higher concentration (Akoumianakis *et al.* 2008). Cadmium is set in the surroundings through various natural and anthropogenic processes like subsurface mining, weathering of rocks, thermal power generation and disproportionately employed waste water and sewage water for irrigation practices in agriculture (Adil *et al.* 2020). Cadmium can alter plant structural and functional properties, inhibit seed germination, root elongation (Ali *et al.* 2014), reduce the final plant height, thereby affecting biomass of the crop plants (Huang *et al.* 2017), and inhibits physiological processes such as relative water contents leading to impairment in plant metabolism (Tang *et al.* 2018). The increase in cadmium concentrations in soil also affects the content of calcium and potassium elements in the leaves of carrot plants (Gadallah 1999).

Various doses of Cd ranging between 0.1 and 10 mM affected early seedling growth of fenugreek seeds and inhibited elongation of both root and hypocotyl (Zayneb *et al.* 2015). The complete germination of dehusked seeds of the indica rice was achieved under aerobic conditions with kinetin (Miyoshi and Sato 1997). GA<sub>3</sub> increased seed germination under abiotic stress in Okra (Ayub 2018). Grain presoaking in kinetin induced remarkable increases in growth parameters under cadmium stress in *Zea mays* (Al-Amri 2019). GA<sub>3</sub> increased length, fresh and dry weight of plant under Cd stress in mung bean (Hassan *et al.* 2021). Presoaking of maize grains with kinetin induced a drastic increase in relative water content (RWC) of maize leaves treated with or without cadmium chloride (Al-Amri 2019). Application of GA<sub>3</sub> alleviated the severe effects of Cd by provoking RWC levels through reduction in metal accumulation (Hasan *et al.* 2020).

Irrigation of soil with cadmium chloride resulted in a reduction in the content of potassium, calcium and increase cadmium in the leaves of maize plants (Al-Amri 2019). GA<sub>3</sub> reduced heavy metal accumulation in plants (Abd EI-Monem *et al.* 2009). The amount of Na<sup>+</sup> significantly reduced when GA<sub>3</sub> and kinetin were applied (Nimir *et al.* 2016). Nitrate accumulation was hampered in *Pisum sativum* under cadmium stress (Harnandez 1997).

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Reports on the effect of exogenous application of kinetin and gibberellic acid on growth, accumulation of ions and its correlations with growth in this plant is still scanty especially with regards to cadmium phytotoxicity in Bangladesh. Therefore, the present investigation was undertaken to examine the effects of exogenously applied kinetin and gibberellic acid on growth, ion transport and its correlations with growth in rice plants under cadmium stress condition.

### Materials and Methods

Rice (*Oryza sativa* L. cv. BRRI-80) seeds were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

Surface sterilized seeds were sown in each earthen pot filled with purified quartz sand. After germination, the pots were kept in the greenhouse, University of Dhaka, exposed to the environmental conditions at a day/night temperature of  $30 \pm 1^\circ\text{C}/25 \pm 1^\circ\text{C}$  and day/night length of 14 hrs/10 hrs. The sand was moistened with half-strength Hoagland solution (Hoagland and Arnon 1950) every 24 hrs. Fourteen days-old seedlings were subjected to  $50 \mu\text{M}$  cadmium made in half-strength Hoagland solution which served as control.  $2 \mu\text{M}$  kinetin mixed with  $50 \mu\text{M}$  cadmium and  $1 \mu\text{M}$   $\text{GA}_3$  mixed with  $50 \mu\text{M}$  cadmium made in half-strength Hoagland solution considered as Treatment 1 (T-1) and Treatment 2 (T-2), respectively. Roots and shoots of plants grown in sand culture were collected at 7, 14, 21 and 28 days of Cd-Kn and Cd- $\text{GA}_3$  treatments. The samples were dried in at  $75^\circ\text{C}$  for 72 hrs. Fresh and dry weight of root and shoot were recorded using a digital balance. The length of both root and shoot was measured with a centimeter scale.

$\text{Na}^+$ ,  $\text{K}^+$  and  $\text{NO}_3^-$  were extracted by boiling the samples with distilled water using water bath. Sodium ( $\text{Na}^+$ ) and Potassium ( $\text{K}^+$ ) ions were measured by a flame photometer (Jenway, PEP-7, UK) at a wavelength of 767 nm and 589 nm, respectively. Nitrate ( $\text{NO}_3^-$ ) was determined following the method of Cataldo *et al.* (1975). Measurement of  $\text{PO}_4^{3-}$  was done according to the method of Jackson (1967). An inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the concentration of cadmium (Cd) in the collected plant samples. RWC was measured according to the method of Barrs and Weatherly (1962).

### Results and Discussion

Under cadmium stress, exogenous application of kinetin ( $2 \mu\text{M}$ ) and gibberellic acid ( $1 \mu\text{M}$ ) was found to be helpful in lengthening the roots and shoots of rice plants. Due to kinetin application the length of root and shoot increased by 26 to 71% and 20 to 50%, respectively, within 7 to 28 days of treatment. Application of  $\text{GA}_3$  also increased length of root and shoot by 41 to 87% and 14 to 37%, respectively, within 7 to 28 days of treatment (Figs. 1a and b). This result indicates that both kinetin and  $\text{GA}_3$  were effective against cadmium stress in case of root and shoot which is in agreement with the findings of Li *et al.* (2018).

Implementation of kinetin and  $\text{GA}_3$  was found to be beneficial in boosting the fresh weight of root and shoot of rice plants under cadmium toxicity. As a result, 77% to 1.6-fold and 79% to 1.2-fold increase in fresh weight of root and shoot were observed for kinetin treatment, respectively, whereas,  $\text{GA}_3$  showed 59% to 1.6-fold and 1.07- to 1.86-fold increment in root and shoot fresh weight, respectively, from 7 to 28 days of treatment (Figs. 2a and b). Similar trend of escalation due to plant growth regulators in the fresh weight was found in summer squash under abiotic stress (Al-Harathi 2021).

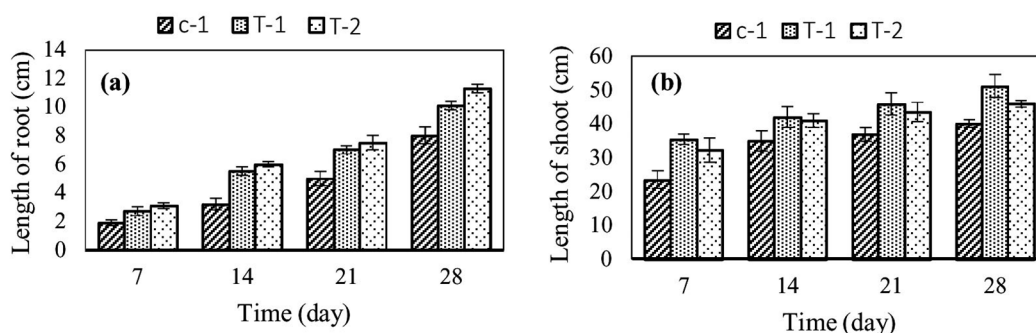


Fig. 1. Effects of kinetin and GA<sub>3</sub> on length of (a) root and (b) shoot of rice plants under cadmium stress (50 μM CdCl<sub>2</sub>.H<sub>2</sub>O) grown in sand culture. C-1 represents 50 μM Cd; T-1 (50 μM Cd + 2 μM kinetin); T-2 (50 μM Cd + 1 μM GA<sub>3</sub>). Each value is the mean of three replicates ± standard error.

Significant escalation in dry weight of root and shoot were observed as for the results of kinetin and GA<sub>3</sub> treatments. Massive increments by 72% to 2-fold and 1.49- to 3.4-fold were observed in case of 2 μM kinetin treatment of root and shoot, respectively, whereas, 44% to 1-fold and 1.7- to 4.5-fold increment were observed in case of 1 μM GA<sub>3</sub> treatment in root and shoot dry weight, respectively, in this regard from the experimental period of 7 to 28 day of treatment (Figs. 2c and d). A dramatic escalation was observed in dry weight of root and shoot of cadmium stressed rice plants, which is in the agreement with the results found for growth regulator enhancing dry weight of three varieties of Gaillardia (El-Kinany *et al.* 2019).

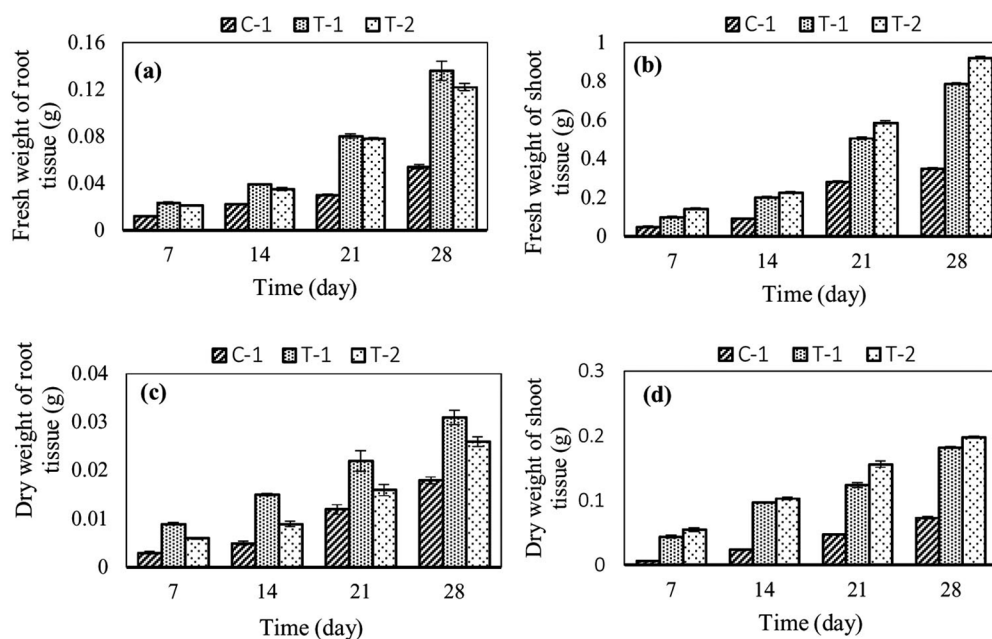


Fig. 2. Effects of kinetin and GA<sub>3</sub> on fresh weight of (a) root and (b) shoot and dry weight of (c) root and (d) shoot of intact rice plants under cadmium stress (50 μM CdCl<sub>2</sub>.H<sub>2</sub>O) grown in sand culture. C-1 represents 50 μM Cd; T-1 (50 μM Cd + 2 μM kinetin); T-2 (50 μM Cd + 1 μM GA<sub>3</sub>). Each value is the mean of three replicates ± standard error.

The external use of kinetin and GA<sub>3</sub> stimulated the Na<sup>+</sup> content in both root and shoot. In root and shoot tissues, Na<sup>+</sup> content reduced by 19 to 36% and 16 to 41%, respectively, for kinetin treatment. On the contrary, GA<sub>3</sub> reduced Na<sup>+</sup> content by 30 to 57% and 12 to 34% in root and shoot tissue, respectively, within 7 to 28 days of treatment (Figs. 3a and b). This result shows the similar trend of reduction in Na<sup>+</sup> content in plants found by Ahanger *et al.* (2018) in salinized *Solanum lycopersicum* due to plant growth regulator.

Kinetin (2 μM) and GA<sub>3</sub> (1 μM) treatments significantly stimulated the quantity of K<sup>+</sup> in both root and shoot of rice plant. In case of root and shoot, 36% to 1-fold and 46% to 1.2-fold of increment were observed for kinetin treatment. Application of GA<sub>3</sub> stimulated K<sup>+</sup> content in root and shoot tissue by 1.6- to 3-fold and 26 to 60%, respectively, within 7 to 28 days of treatment (Figs. 3c and d). These results showed analogy with the findings of Abou-El-Ghait *et al.* (2018) in *Dendranthema grandiflorum* cv. Art Queen plants in case of plant growth regulators.

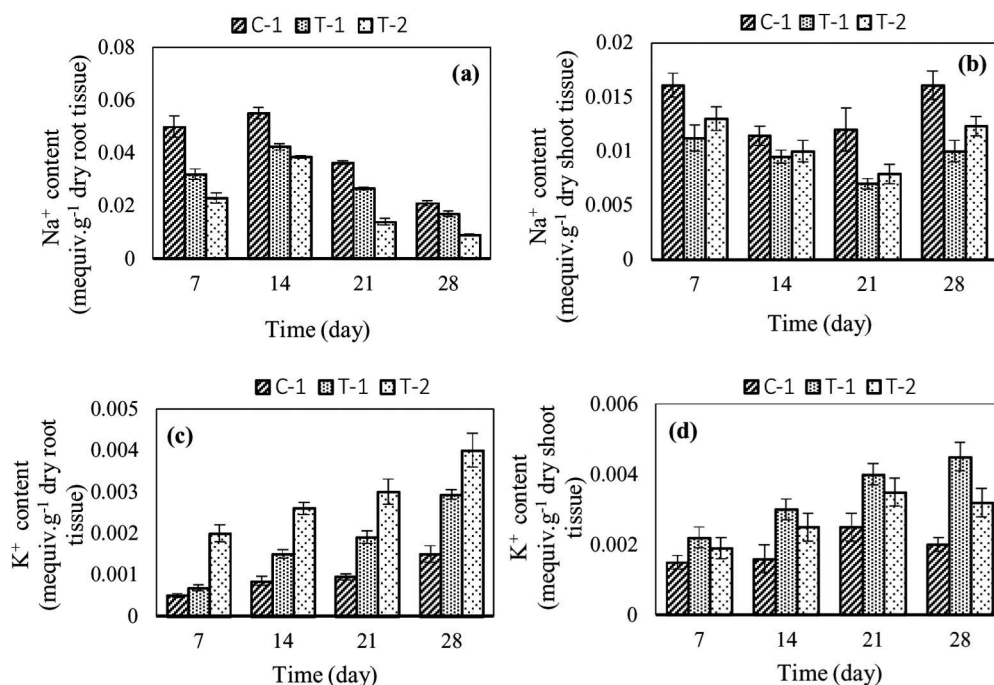


Fig. 3. Effects of kinetin and GA<sub>3</sub> on Na<sup>+</sup> content of (a) root and (b) shoot and K<sup>+</sup> content of (c) root and (d) shoot of rice plants under cadmium stress (50 μM CdCl<sub>2</sub>.H<sub>2</sub>O) grown in sand culture. C-1 represents 50 μM Cd; T-1 (50 μM Cd + 2 μM kinetin); T-2 (50 μM Cd + 1 μM GA<sub>3</sub>). Each value is the mean of three replicates ± standard error.

Application of kinetin (2 μM) and GA<sub>3</sub> (1 μM) treatments decreased the Cd<sup>2+</sup> content in both root and shoot. In root and shoot tissue, Cd<sup>2+</sup> content reduced by 15 to 16.2% and 13 to 33% for 2 μM kinetin treatment, respectively. On the contrary, GA<sub>3</sub> reduced Cd<sup>2+</sup> content by 43 to 50% and 25 to 54% in root and shoot tissue, respectively, within 7 to 28 days of treatment (Figs. 4a and b). Both kinetin and GA<sub>3</sub> decreased Cd uptake in root and shoot under cadmium stress in rice plants, which are in accordance with the findings of Wang *et al.* (2015) in maize.

The exogenous application of kinetin and GA<sub>3</sub> treatments stimulated the NO<sub>3</sub><sup>-</sup> content in both root and shoot. In root and shoot tissue, NO<sub>3</sub><sup>-</sup> content increased by 42% to 1.2-fold and 51% to 1.4-fold, respectively, for 2 μM kinetin treatment. On the contrary, GA<sub>3</sub> increased NO<sub>3</sub><sup>-</sup> content by 94% to 1.8- fold and 25% to 1.3-fold in root and shoot, respectively, within 7 to 28 days of treatment (Figs. 5a and b). Growth regulator caused accumulation of NO<sub>3</sub><sup>-</sup> content at a huge amount in both root and shoot of cadmium stressed rice seedlings which correlates with the trend observed in *Chamomile recutita* L. Rausch (Reda *et al.* 2010).

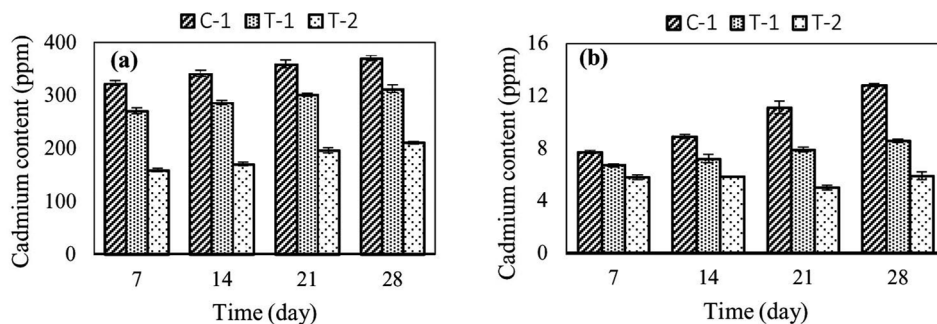


Fig. 4. The effect of kinetin and GA<sub>3</sub> on Cd<sup>2+</sup> content of (a) root and (b) shoot of rice plants under cadmium stress (50 μM CdCl<sub>2</sub>.H<sub>2</sub>O) grown in sand culture. C-1 represents 50 μM Cd; T-1 (50 μM Cd + 2 μM kinetin); T-2 (50 μM Cd + 1 μM GA<sub>3</sub>). Each value is the mean of three replicates ± standard error.

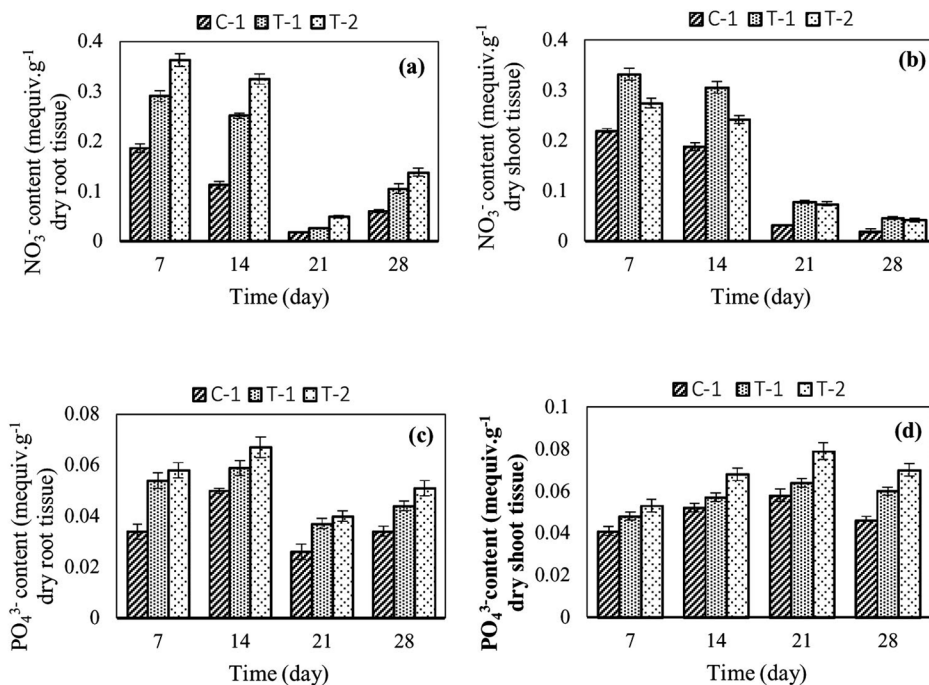


Fig. 5. Effects of kinetin and GA<sub>3</sub> on NO<sub>3</sub><sup>-</sup> content of (a) root and (b) shoot and PO<sub>4</sub><sup>3-</sup> content of (c) root and (d) shoot of rice plants under cadmium stress (50 μM CdCl<sub>2</sub>.H<sub>2</sub>O) grown in sand culture. C-1 represents 50 μM Cd; T-1 (50 μM Cd + 2 μM kinetin); T-2 (50 μM Cd + 1 μM GA<sub>3</sub>). Each value is the mean of three replicates ± standard error.

Kinetin and GA<sub>3</sub> treatments significantly stimulated the quantity of PO<sub>4</sub><sup>3-</sup> in both root and shoot of the rice plants. For kinetin treatment, PO<sub>4</sub><sup>3-</sup> content was increased by 18 to 58% and 9 to 30% in root and shoot tissue, respectively, and application of GA<sub>3</sub> stimulated PO<sub>4</sub><sup>3-</sup> content in both root and shoot by 34 to 70% and 29 to 52%, respectively, (Figs. 5c and d). Both the hormones were found effective in enhancing the PO<sub>4</sub><sup>3-</sup> content in root and shoot, similar trend was found by Abd El-Aal (2014) regarding the PO<sub>4</sub><sup>3-</sup> content in *Hippeastrum vittatum*.

Under cadmium stress, exogenous administration of kinetin and GA<sub>3</sub> were found feasible for increasing relative water content ranged from 20 to 29% and 14 to 25%, respectively, in shoot tissue (Fig. 6). Similar trends were reported in case of kinetin treatment in *Sorghum bicolor* (Gadallah and Sayed 2001) and in case of GA<sub>3</sub> treatments in summer squash (Al-Harathi *et al.* 2021) under abiotic stress.

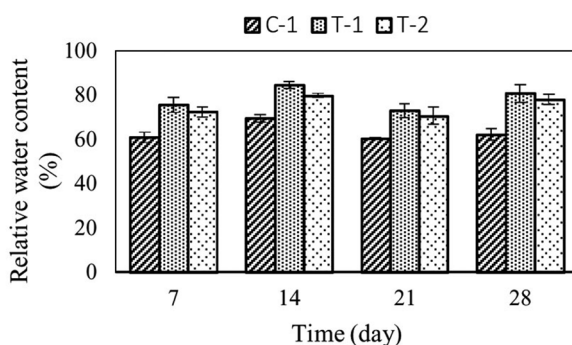


Fig. 6. Effects of kinetin and GA<sub>3</sub> on relative water content of shoot of rice plants under cadmium stress (50 μM CdCl<sub>2</sub>.H<sub>2</sub>O) grown in sand culture. C-1 represents 50 μM Cd; T-1 (50 μM Cd + 2 μM kinetin); T-2 (50 μM Cd + 1 μM GA<sub>3</sub>). Each value is the mean of three replicates ± standard error.

Application of kinetin and GA<sub>3</sub> to rice plants was found to be highly promising in reducing cadmium content in both root and shoot, increasing plant length, fresh and dry weight of root and shoot, ionic balance and relative water content in comparison to Cd stressed plants. The uncontrolled use of synthetic fertilizers in agricultural field is subjecting plants to uptake Cd to a greater extent, ultimately entering the food chain. Therefore, finding mitigation strategies to reduce Cd uptake is crucial. Kinetin and gibberellic acid were found to ensure restricted Cd entry into the plants, making it a cost effective and easy way to combat the serious Cd threat. The concentrations used of both kinetin and GA<sub>3</sub> in the present study were very low, so very little amount of both plant growth regulators was used that have shown good results in terms of counteracting the negative effects of cadmium on rice plants.

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