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Short Communication

Effects of Calcium on the Alleviation of Cadmium Toxicity in *Salix matsudana* and Its Effects on Other Minerals

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Abstract

Effects of exogenous calcium (5 mmol/L) on accumulation of manganese, iron, copper and zinc in different organs (roots, new stems, leaves and old stems) of *Salix matsudana* Koidz exposed to 10 and 50 μmol/L cadmium for 7, 14, 21 and 28 d were investigated in order to further understand calcium effects in alleviating cadmium toxicity. The results showed that cadmium could inhibit seedling growth of *S. matsudana*. 5 mmol/L calcium had a alleviating toxic effects on seedling growth. Results indicated that cadmium contents in the different organs increased significantly ($P<0.05$) with increasing cadmium concentration and prolonged duration of treatment. Cadmium concentrated mainly in the roots, and small amounts were transferred to shoots. 5 mmol/L calcium decreased the cadmium levels significantly ($P<0.05$) in the organs of *S. matsudana* exposed to all the cadmium concentrations used during the whole experiment. Data revealed that cadmium stress mainly affected the accumulation of manganese both in roots and shoots. Cadmium could induce the high levels of iron, zinc and copper in roots. Data also indicated that exogenous calcium could promote the absorption of copper, iron, zinc and manganese to different extent, suggesting that calcium has the alleviating effect on the toxicity of cadmium.

Keywords: cadmium (Cd), calcium(Ca), minerals, *Salix matsudana* Koidz

Introduction

Cadmium (Cd) is a highly toxic trace element mainly derived from industrial processes and phosphate fertilizers [1]. Cd concentrations in soil near smelters are extremely high, up to 200 mg/kg [2]. Considerable attention has been attached to problems associated with Cd pollution, with the development of modern industry and agriculture. It is a non-essential heavy metal with high toxicity to plants, animals and humans [3] and has been regarded as most dangerous environmental pollutant [4]. Cd is of great concern because it is a nonessential toxic element which has toxic effects on plants, animals and humans with a very small dose [5]. Under Cd stress, growth and development of plants are affected, such as inhibiting photosynthesis and respiration, disturbing the normal division of root tip cells, and causing imbalances in nutrition

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[6-11]. Therefore, remediation of Cd pollution in the environment to avoid its threats is very important and urgently needed [5].

Some heavy metals (HMs), such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), are required for the normal growth, development, and reproduction of plants [12]. Cd interferes with the uptake, transport, and use of different macro- and micronutrients. Microelements Zn, Fe, Mn, and Cu interfering with Cd uptake may decrease Cd concentrations in plants [13]. Calcium (Ca) is known as a necessary nutrient for plant growth and development. As the second messenger Ca can couple extracellular signals with intracellular physiological and biochemical reactions, enhancing plant resistance to environmental stress by stabilizing cell wall, cell membrane structure and inducing expression of specific genes [14, 15]. It was reported that exogenous Ca is an important signaling messenger and essential nutrient element and plays an important role in many adaptation and development processes of plant cells [16].

Salix matsudana Koidz (Chinese willow) grows in a wide range of climatic conditions and is one of the most widely cultivated willow species in China [17]. It is a fast-growing, productive, and deeply rooted tree of the willow species that adapts to temperate region climatic conditions and has the ability to tolerate high Cd [18, 19]. These traits make it a potential ideal candidate for phytoremediation of Cd-contaminated waters and soils [18, 20]. Some research works were reported to be involved in Cd stress on mineral uptake and accumulation in *S. matsudana* [7, 20, 21]. However, the research on the effects of Ca on other metal accumulation and translocation in *S. matsudana* under Cd stress remains unknown. How Ca helps to alleviate the Cd-induced toxicity requires more investigations. Therefore, *S. matsudana* was used to explore the alleviating effect of Ca and its specific function during the uptake and accumulation of Cd and other minerals (Cu, Zn, Mn and Fe) in roots and shoots of *S. matsudana* in the present investigation.

Experimental

Seedlings Cultivation and Treatment

Woody cuttings (15 cm long) from one-year-old shoots of *S. matsudana* grown in the campus of Tianjin Normal University, China were collected and rooted in plastic buckets filled with distilled water for two weeks under glasshouse conditions of 23±5ºC and 55±10% relative humidity before starting the experiments. After the seedlings burgeoned, healthy (without leaf chlorotic spots and withered) and uniform (similar height) plants were transferred and cultivated to 1/2 Hoagland nutrient solution for 7, 14, 21 and 28 d. Cd and Ca were added to the containers to form five treatments: basal nutrient solution (control, without Cd and Ca), 10 μmol/L Cd, 10 μmol/L Cd and 5 mmol/L Ca, 50 μmol/L Cd, 50 μmol/L Cd and 5 mmol/L Ca. The nutrient solution consisted of 5 mmol/L KNO_3, 5 mmol/L $Ca(NO_3)_2$, 1 mmol/L KH_2PO_4, 50 μmol/L H_3BO_3, 10 μmol/L FeEDTA, 4.5 μmol/L $MnCl_2$, 3.8 μmol/L $ZnSO_4$, 0.3 μmol/L $CuSO_4$, and 0.1 μmol/L $(NH_4)_6Mo_7O_{24}$,

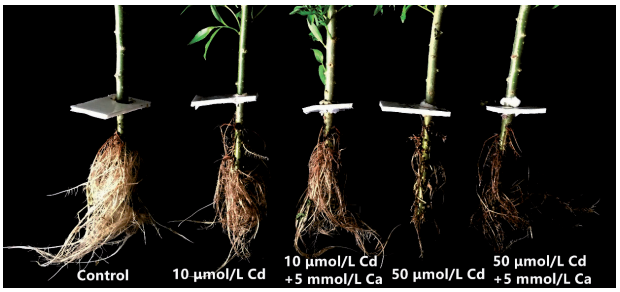

Fig. 1 Effect of exogenous Ca on the growth of *S. matsudana* under Cd stress (28d).

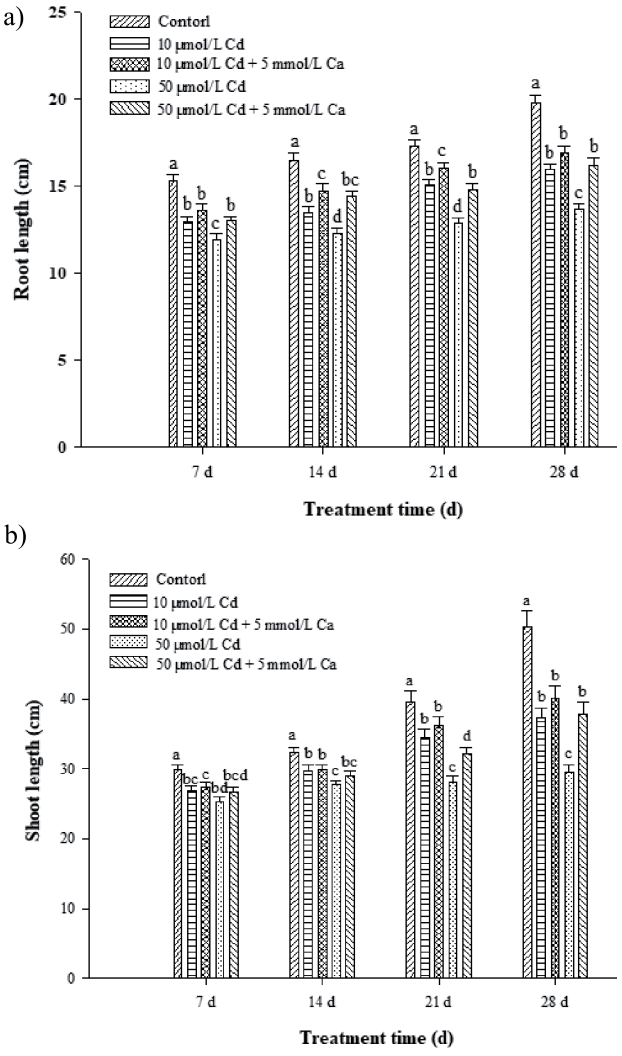

Fig. 2 Effect of exogenous Ca on plant height and root length of *S. matsudana* under Cd stress.
Values followed by different letters differ significantly from each other (P<0.05, t-test). Means±SE, n = 5.

Table 1. Effect of exogenous Ca on Cd content in different organs of *S. matsudana* under different concentrations of Cd sress.

Time (d)	Treatment Cd (μmol/L) Ca (mmol/L)	Cd (μg/g, DW±SE)			
		Root	New stem	Leaf	Old stem
7	0	4.70±0.46a	4.85±0.01a	5.86±0.01a	1.19±0.01a
	10Cd	584.55±1.58b	68.54±0.22b	58.27±0.12b	6.98±0.07b
	10Cd+5Ca	571.57±2.32c	36.80±0.09c	22.38±0.07c	2.68±0.01c
	50Cd	2041.95±4.04d	71.35±0.23d	23.54±0.03d	9.38±0.01d
	50Cd+5Ca	963.50±6.74e	63.36±1.92b	47.81±0.32b	5.08±0.02e
14	0	4.97±0.01a	5.67±0.08a	6.27±0.01a	1.28±0.04a
	10Cd	1021.37±1.56b	70.13±0.10b	57.22±0.73b	8.38±0.02b
	10Cd+5Ca	879.55±5.57c	40.38±1.21c	27.99±0.02c	4.98±0.02c
	50Cd	2563.91±0.85d	151.57±2.45d	103.83±0.03d	17.57±0.05d
	50Cd+5Ca	1329.55±1.24e	121.89±0.71e	83.24±2.52e	14.77±0.06e
21	0	5.00±0.02a	5.76±0.04a	6.93±0.02a	1.30±0.01a
	10Cd	1360.18±1.62b	112.16±1.39b	134.88±0.35b	15.22±0.07b
	10Cd+5Ca	1071.39±31.06c	52.45±0.08c	62.10±0.20c	6.46±0.06c
	50Cd	2982.71±1.38d	171.16±0.53d	167.46±0.06d	25.74±0.09d
	50Cd+5Ca	2073.87±3.73e	145.01±0.31e	149.85±0.49e	20.93±0.56e
28	0	5.89±0.01a	5.81±0.01a	7.11±0.00a	1.48±0.07a
	10Cd	1365.4±0.44b	105.46±1.75b	174.93±0.21b	28.04±0.01b
	10Cd+5Ca	1327.20±0.22c	97.83±0.08c	124.68±2.29c	20.9±1.22c
	50Cd	3422.11±3.59d	223±0.57d	188.24±1.44d	56.74±0.08d
	50Cd+5Ca	3234.00±1.17e	206.89±0.42e	166.54±0.57e	41.53±1.08e

Values followed by different letters differ significantly from each other ($P<0.05$, *t*-test). Means±SE, n = 3.

adjusted to pH 5.5. Cd was provided as cadmium chloride ($CdCl_2 \times 2.5\ H_2O$). Cadmium stock solution was prepared in deionized water. Ca was provided as calcium chloride ($CaCl_2$). The nutrient solutions were renewed once every 7 d, and the air pump was used for ventilation during the culture. All treatments were performed in three replicates.

Determination of Cd and Other Minerals

Roots and shoots from each treatment were harvested from the cuttings at each time interval (7 d). After removal of necrotic and putrid tissue, the shoots were washed thoroughly with running tap water for 30 min and then with deionized water to remove traces of nutrients, Cd and Ca ions from seedling surfaces. The plants were divided root, leaf, new stem and old stem. The samples were dried for 3 d at 45ºC, 1 d at 80ºC, and 12 h at constant 105ºC in an oven. All dried plant samples were prepared using wet-digestion methods. The contents of Cd, Fe, Mn, Cu and Zn were analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES) (Leeman Labs Inc., USA).

Statistical Analysis

All statistical values were calculated using the Statistical Package for Social Sciences (SPSS) program release 17.0 for Windows (SPSS, Chicago, IL, USA) and Sigma Plot 12.5 using means±standard error (SE). For equality of averages the *t*-test was applied. Results were considered statistically significant at $P<0.05$.

Results

Effects of Exogenous Ca on the Growth of *S. matsudana* under Cd Stress

Effects of Cd on Root and Shoot Growth

The effects of Cd on root growth of *S. matsudana* varied with the concentration and duration of treatment (Figs 1 and 2). 10 and 50 μmol/L Cd exposure had toxic effect on root growth and the shoot length was inhibited significantly with increasing treatment time when compared to control ($P<0.05$). The shoot growth treated

Table 2. Effect of exogenous Ca on Mn content in different organs of *S. matsudana* under different concentrations of Cd sress.

Time (d)	Treatment Cd (μmol/L) Ca (mmol/L)	Mn (μg/g, DW±SE)			
		Root	New stem	Leaf	Old stem
7	0	67.22±0.01a	14.48±0.01a	74.14±0.20a	4.19±0.01a
	10Cd	32.28±0.12b	9.39±0.04b	70.93±0.08b	2.18±0.03b
	10Cd+5Ca	21.29±0.19c	10.64±0.03c	77.18±0.25c	3.35±0.01c
	50Cd	28.50±0.03d	11.06±0.05c	65.16±0.08d	3.15±0.02d
	50Cd+5Ca	45.64±0.11e	13.39±0.23d	68.94±0.36e	4.02±0.02e
14	0	150.57±0.21a	15.67±0.16a	79.69±0.15a	4.27±0.08a
	10Cd	51.03±0.03b	8.4±0.02b	68.05±0.23b	2.96±0.01b
	10Cd+5Ca	30.89±0.22c	8.81±0.30b	72.25±0.06c	3.61±0.01c
	50Cd	17.57±0.01d	10.59±0.03c	60.62±0.03d	3.11±0.01b
	50Cd+5Ca	39.69±0.05e	12.54±0.25d	65.86±0.17e	4.57±0.40a
21	0	152.33±0.26a	16.80±0.03a	89.12±0.12a	4.78±0.01a
	10Cd	63.19±0.15b	6.8±0.06b	60.80±0.24b	3.15±0.01b
	10Cd+5Ca	44.66±0.17c	7.94±0.02c	67.34±0.12c	3.95±0.04c
	50Cd	14.23±0.01d	8.15±0.03c	55.96±0.07d	3.41±0.02d
	50Cd+5Ca	24.24±0.03e	9.83±0.21d	58.94±0.22e	4.71±0.11a
28	0	239.28±0.19a	17.38±0.01a	90.34±0.39a	4.84±0.21a
	10Cd	90.01±0.11b	6.16±0.11b	57.98±0.01b	3.35±0.02b
	10Cd+5Ca	53.1±0.15c	6.70±0.02c	63.04±0.16c	4.38±0.16c
	50Cd	12.95±0.01d	7.42±0.08d	49.25±0.24d	3.56±0.01b
	50Cd+5Ca	20.28±0.05e	8.55±0.02e	56.79±0.11e	4.89±0.15a

Values followed by different letters differ significantly from each other ($P<0.05$, *t*-test). Means±SE, n = 3.

with 10 and 50 μmol/L Cd showed the same growth trend as control. The effects of Cd on the morphology of the roots of *S. matsudana* exposed to 10 and 50 μmol/L Cd showed to be dark brown in colour when compared with control after Cd treatment for 28 d.

Effects of Exogenous Ca on Root and Shoot Growth under Cd Stress

The effect of Ca on root growth of *S. matsudana* exposed to Cd varied with the concentration and duration of treatment (Fig. 1). 5 mmol/L Ca had the alleviating toxic effects of Cd on root growth induced by Cd significantly ($P<0.05$) in comparison with the groups exposed to 10 and 50 μmol/L Cd during the whole experiment, except for the group treated with 10 μmol/L Cd for 7 d. As for shoot length, 5 mmol/L Ca also had alleviating toxic effects significantly ($P<0.05$) on shoot length treated with 50 μmol/L Cd for 21 and 28 d.

Effects of Exogenous Ca on Cd Accumulation and Other Minerals

Cd Content

Cd uptake and accumulation in the different organs of *S. matsudana* varied depending on Cd concentration and treatment time. Data showed that the Cd contents in roots exposed to 10 and 50 μmol/L Cd increased significantly ($P<0.05$) with increasing Cd concentration and prolonged duration of treatment when compared to control (Table 1). The Cd contents in new stem, leaf and old stem of *S. matsudana* had the same trend as roots. The levels of Cd in the different organs of *S. matsudana* were in the order as follows: root > new stem > leaf > old stem during 14 d treatment period. Cd concentrated mainly in the roots, and small amounts were transferred to shoots. Data here indicated that 5 mmol/L Ca could decrease the Cd levels significantly ($P<0.05$) in roots, new stems, leave and old stems of *S. matsudana* exposed to all Cd concentrations used during the whole experiment.

Table 3. Effect of exogenous Ca on Fe content in different organs of *S. matsudana* under different concentrations of Cd sress.

Time (d)	Treatment Cd(μmol/L) Ca(mmol/L)	Fe (μg/g, DW±SE)			
		Root	New stem	Leaf	Old stem
7	0	127.25±0.2a	50.69±0.19a	128.8±0.68a	35.13±0.13a
	10Cd	339.94±0.11b	37.22±0.04b	88.97±0.40b	23.02±0.34b
	10Cd+5Ca	279.13±0.23c	45.79±0.18c	68.30±0.40c	28.73±0.07c
	50Cd	307.09±0.12d	43.94±0.50d	72.36±0.11d	48.00±0.19d
	50Cd+5Ca	370.95±0.14e	50.43±0.13a	98.95±0.5e	55.73±0.29e
14	0	254.31±0.14a	49.1±0.01a	120.55±0.56a	19.68±0.11a
	10Cd	487.21±0.21b	29.87±0.17b	77.06±0.16b	17.76±0.13b
	10Cd+5Ca	374.03±0.20c	33.59±0.56c	62.60±0.24c	24.58±0.04c
	50Cd	498.78±0.23d	32.79±0.13d	69.85±0.17d	33.99±0.20d
	50Cd+5Ca	512.98±0.22e	46.66±0.64e	86.47±0.02e	40.71±0.04e
21	0	267.93±0.45a	44.5±0.14a	112.24±0.46a	17.53±0.04a
	10Cd	570.17±0.56b	24.68±0.07b	63.13±0.31b	15.58±0.13b
	10Cd+5Ca	455.58±0.11c	30.74±0.21c	51.48±0.13c	20.90±0.27c
	50Cd	580.53±0.52d	25.16±0.25d	65.27±0.19d	25.13±0.13d
	50Cd+5Ca	613.97±0.54e	32.78±0.21e	83.68±0.45e	34.68±0.28e
28	0	270.68±0.39a	38.26±0.11a	102.68±0.43a	15.58±0.29a
	10Cd	628.1±0.61b	13.01±0.25b	53.63±0.08b	3.20±0.03b
	10Cd+5Ca	546.49±0.06c	22.99±0.08c	40.98±0.24c	6.89±0.22c
	50Cd	765.49±0.64d	17.84±0.32d	63.47±0.46d	10.79±0.28d
	50Cd+5Ca	827.31±0.15e	24.13±0.12e	74.64±0.15e	17.67±0.01e

Values followed by different letters differ significantly from each other ($P<0.05$, *t*-test). Means±SE, n = 3.

Mn Content

Statistical analysis showed the presence of significant correlations among the concentrations of Cd and microelements (Ca and Mn). The accumulation of Mn in *S. matsudana* roots, new stems, leaves and old stems varied with Cd concentration and treatment time. As shown in Table 2, in the presence of Cd, the contents of Mn in roots, new stems, leaves and old stems of *S. matsudana* decreased significantly (*P<0.05*) when compared to control. 5 mmol/L Ca was found to increase Mn accumulation significantly (*P<0.05*) in the different of *S. matsudana* stressed by Cd during the whole experiment in comparison with the Cd-treated groups (without Ca) except for the root groups exposed to 10 μmol/L Cd + 5 mmol/L Ca for 7, 14, 21 and 28 d. Data also showed that 5 mmol/L Ca could not promote the absorption of Mn in the roots treated with 10 μmol/L Cd during the duration of the experiment.

Fe Content

The contents of Fe in roots exposed to 10 and 50 μmol/L Cd during the whole experiment were high significantly (*P<0.05*) when compared to control (Table 3). However, the contents of Fe in new stems, leave and old stems of *S. matsudana* exposed to all Cd concentrations used during the whole experiment were observed to be low significantly (*P<0.05*) in comparison with control. Effects of exogenous Ca (5 mmol/L) on Fe accumulation of *S. matsudana* varied with the Cd concentration and the organs of *S. matsudana*. Data from Table 3 revealed that 5 mmol/L Ca promoted the Fe uptake significantly (*P<0.05*) in the roots exposed 50 μmol/L Cd during the whole experiment. In the other organs (new stems, leaves and old stems), the exogenous Ca increased Fe accumulation in comparison with the groups treated with all Cd concentrations used except for the leaf groups exposed to 10 μmol/L Cd for 7, 14, 21 and 28 d.

Table 4. Effect of exogenous Ca on Zn content in different organs of *S. matsudana* under different concentrations of Cd sress.

Time (d)	Treatment Cd(μmol/L) Ca(mmol/L)	Zn (μg/g, DW±SE)			
		Root	New stem	Leaf	Old stem
7	0	152.26±0.88a	181.31±0.17a	146.79±0.22a	28.75±0.04a
	10Cd	237.26±0.07b	131.92±0.14b	109.76±0.10b	29.93±0.14b
	10Cd+5Ca	304.28±0.23c	161.23±0.23c	117.47±0.30c	30.35±0.05c
	50Cd	196.09±0.28d	103.10±0.67d	100.61±0.09d	27.77±0.05d
	50Cd+5Ca	255.07±1.02e	154.85±0.15e	127.00±0.29e	29.07±0.09e
14	0	140.74±0.63a	147.73±0.26a	156.65±0.23a	26.12±0.26a
	10Cd	224.94±0.28b	126.72±0.40b	126.92±0.18b	27.05±0.01b
	10Cd+5Ca	273.41±0.61c	134.48±0.22c	146.42±0.31c	29.95±0.16c
	50Cd	173.87±0.37d	98.52±0.22d	116.17±0.17d	25.44±0.12d
	50Cd+5Ca	238.25±0.48e	120.06±0.06e	149.44±0.14e	27.68±0.01e
21	0	131.85±0.19a	129.57±0.17a	171.67±0.27a	25.14±0.10a
	10Cd	212.96±0.17b	97.65±0.12b	143.56±0.15b	26.23±0.10b
	10Cd+5Ca	242.64±0.15c	112.15±0.17c	163.62±0.19c	28.09±0.23c
	50Cd	164.84±0.18d	84.97±0.35d	121.39±0.22d	18.73±0.13d
	50Cd+5Ca	201.94±0.64e	97.05±0.47e	156.60±0.64e	22.28±0.23e
28	0	87.50±0.11a	109.86±0.23a	180.91±0.44a	24.00±0.09a
	10Cd	201.58±0.24b	87.39±0.18b	168.07±0.35b	24.76±0.04b
	10Cd+5Ca	230.96±0.24c	91.87±0.25b	175.95±0.24c	25.66±0.19c
	50Cd	141.89±0.20d	80.68±0.34c	141.18±0.46d	16.84±0.10d
	50Cd+5Ca	196.53±0.29e	84.39±0.01b	171.30±0.32e	20.40±0.28e

Values followed by different letters differ significantly from each other ($P<0.05$, *t*-test). Means±SE, n = 3.

Zn Content

The Zn contents in different organs of *S. matsudana* were examined. The effects of Cd on Zn uptake and accumulation are shown in Table 4. The results indicated that Zn contents increased significantly (*P<0.05*) in the roots exposed to 10 and 50 μmol/L Cd and the old stems treated with 10 μmol/L Cd during the whole experiment when compared with control. But Zn levels in new stems and leaves stressed by 10 and 50 μmol/L Cd and the old stems exposed to 50 μmol/L Cd decreased significantly (*P<0.05*). Table 4 showed that the exogenous Ca (5 mmol/L) could promote the Zn contents significantly (*P<0.05*) in all organs of *S. matsudana* exposed to Cd during the whole experiment time versus controls (the organs treated with Cd but not adding exogenous Ca).

Cu Content

Effects of Cd on Cu levels in roots, new stems, leaves and old stems of *S. matsudana* varied with the different concentration of Cd and duration of treatment (Table 5). The Cu levels in roots exposed to 50 μmol/L Cd during the whole experiment time were observed to be high significantly (*P<0.05*) in comparison with control, but the Cu levels in roots treated with 10 μmol/L Cd decreased significantly (Table 5). As compared with control, the contents of Cu in new stem, leaf and old stem exposed to 10 μmol/L Cd increased significantly (*P<0.05*) during the whole treatment time. But, at high Cd concentration (50 μmol/L), the Cu contents in new stem and old stem were noted to be low significantly (*P<0.05*), except for the new stem treated with Cd for 7 d. Data from Table 5 also revealed that the level of Cu in the leaf groups exposed to 50 μmol/L Cd was noted to be high significantly when compared with control during whole experimental cycle, except for the 28th d. The effects of Ca on Cu concentration in the organs of *S. matsudana* under Cd stress are presented in Table 5. The Cu contents in roots exposed to 10 μmol/L Cd were noted to be high significantly (*P<0.05*) during the duration of the experiment, but the contents in roots treated with 50 μmol/L Cd decreased significantly. The exogenous Ca (5 mmol/L) showed the decreasing trend in the contents of new stems exposed to

Table 5. Effect of exogenous Ca on Cu content in different organs of *S. matsudana* under different concentrations of Cd sress.

Time (d)	Treatment Cd(μmol/L) Ca(mmol/L)	Cu (μg/g, DW±SE)			
		Root	New stem	Leaf	Old stem
7	0	28.52±0.08a	26.02±0.11a	13.64±0.10a	18.78±0.10a
	10Cd	13.05±0.16b	35.39±0.19b	39.33±0.13b	25.91±0.32b
	10Cd+5Ca	41.51±0.08c	35.35±0.23b	36.18±0.28c	25.56±0.17b
	50Cd	36.01±0.19d	25.73±0.12a	22.43±0.10d	16.80±0.26c
	50Cd+5Ca	27.08±0.13e	18.16±0.24c	11.5±0.09e	9.35±0.04d
14	0	30.95±0.14a	27.44±0.12a	16.15±0.12a	19.51±0.18a
	10Cd	17.86±0.09b	36.15±0.19b	38.54±0.25b	23.99±0.12b
	10Cd+5Ca	48.40±0.09c	36.36±0.07b	33.59±0.02c	24.70±0.14c
	50Cd	51.04±0.13d	24.41±0.28c	22.6±0.04d	15.74±0.03d
	50Cd+5Ca	39.8±0.03e	13.65±0.04d	10.52±0.09e	8.94±0.21e
21	0	31.28±0.15a	28.42±0.12a	21.23±0.24a	20.13±0.08a
	10Cd	21.45±0.04b	30.78±0.25b	36.80±0.08b	23.49±0.14b
	10Cd+5Ca	51.89±0.09c	30.65±0.08b	32.91±0.07c	24.28±0.13c
	50Cd	61.11±0.14d	23.8±0.13c	21.91±0.16d	16.70±0.12d
	50Cd+5Ca	49.92±0.15e	11.71±0.01d	9.95±0.23e	8.20±0.22e
28	0	32.46±0.03a	28.86±0.27a	22.15±0.19a	23.81±0.12a
	10Cd	32.09±0.15b	33.24±0.15b	35.70±0.30b	23.51±0.10b
	10Cd+5Ca	55.74±0.01c	33.63±0.18b	32.33±0.04c	24.68±0.02c
	50Cd	81.78±0.16d	22.34±0.12c	20.13±0.14d	16.15±0.17d
	50Cd+5Ca	61.61±0.10e	9.56±0.21d	9.32±0.12e	8.82±0.22e

Values followed by different letters differ significantly from each other ($P<0.05$, *t*-test). Means±SE, n = 3.

50 μmol/LCd, while there was no significant difference at 10 μmol/L Cd. The levels of Cu in leaves of *S. matsudana* stressed by Cd had a decreasing trend with increasing Cd concentration and duration of treatment. Data from Table 5 also showed that the exogenous Ca could induce the significantly low contents of old stems stressed with 50 μmol/L Cd.

Discussion

Accumulation of Cd often results in visible plant symptoms, such as stunted growth, leaf chlorosis, browning of roots and alteration in the activities of many key enzymes in various metabolic pathways [19, 22 , 23]. In the present investigation, reductions in root and shoot length of *S. matsudana* were observed with increasing Cd concentrations. The effect of Cd on root lengths reduction of *S. matsudana* was more remarkable than that of shoots, because the root was the organ directly exposed to Cd, which were consistent with earlier findings by Stravin-skienė and Račaitė [24] in *Trifolium repens* L. *S. matsudana* has the ability to accumulate Cd primarily in its roots, and transport and concentrate it in its stems and levels in much lesser concentrations. The distribution may be due to the mobilization of the protective mechanisms of plants, which inhibits the transport to further tissues and organs [22]. The results obtained here are consistent with earlier findings [20-22, 25, 26].

Ca is an essential plant macronutrient that involved in various plant physiological processes, such as plant growth and development, cell division, cytoplasmic streaming, photosynthesis and intracellular signaling transduction [23, 27, 28]. Some research works demonstrated that Ca could regulate a range of activities within the cell, such as cell division and elongation, cytoplasmic streaming, photomorphogenesis and plant defense against environmental stresses [29-33]. Several reports indicated that Ca could compete with Cd in plants to increase the tolerance of plants to heavy metals and reduce the damage caused by heavy metals to plants [34, 35]. Ca alleviates Cd-induced oxidative stress in plants by scavenging reactive oxygen species (ROS), increasing antioxidant levels and enhancing antioxidant enzymes activities [28]. Cd and Ca have similar ionic

radii (0.099 nm and 0.097 nm, respectively) and compete for the same Ca channels in plants [36]. Shortly after Cd enters the cytoplasm, it binds to certain sites in the root tip apoplast, affecting the function of the plasma lemma pumps transporting Ca ions and resulting in the interference of Ca uptake [7]. The results here showed that the exogenous 5 mmol/L Ca could decrease the Cd levels significantly ($P<0.05$) in the organs of *S. matsudana* exposed to all Cd concentrations used during the whole experiment, revealing that Ca had the alleviating toxic effects on the seedling growth stressed by Cd. Previous studies indicated that the increase of Ca concentration in roots of *Brassica juncea* [37] and *Hordeum vulgare* [38] under Cd stress was a potential mechanism for reducing the toxic effects of Cd. The phenomenon may possibly be explained by the fact that when Ca and Cd coexist in the adsorption system, Ca can reduce the adsorption of Cd, thereby reduce the toxic effect of Cd on plants [39]. Xu et al. [27] also showed that as with the essential micronutrients, Cd was taken up by plants due its physicochemical similarity to other cations (such as Ca); consequently, Cd could compete with Ca and enter the plant through transport systems meant for Ca. The results demonstrated that low concentration of Ca treatment also enhanced the uptake of Cd in the roots of *Brassica juncea*, *Sesbania sesban* and *Boehmeria nivea* [40, 41], suggesting increased the phytotoxicity of Cd. Other studies pointed out that plant treated with heavy metals often increases suberin and lignin contents [42]. Studies conducted by Baxter et al., [43] and Zeng et al. [23] indicated that lignin was mainly found in the cell wall of the inner and outer cortex of the roots, and also played an important role to regulate the absorption of water and mineral elements. Under Cd stress, the expression of genes was involved in lignin synthesis. As lignin accumulates, root cell membrane integrity is lost in Cd-treated plants, resulting in the adherence of Cd to its root surface stronger when compared with Cd+Ca-treated plants. In this way the growth of primary root in Cd-treated plants is inhibited more severely [23].

Cu, Fe, Mn and Zn are metal micronutrients that play an essential role in plant cell growth and development [44]. Dynamic equilibrium of these heavy metals in plant cells is necessary to avoid deficiency and toxicity [13]. As indicated by Liu et al. [45], the interactions of Cd and Fe, Cu and Zn were synergetic in uptake and translocation from root to shoot by rice plants. It well demonstrated that Cd could alter the uptake of minerals by plants through its effects on the availability of minerals from the soil. Cd toxicity may affect plant metabolism, disturbing the accumulation and translocation of mineral nutrient. Plants have a complex metal homeostasis network system that regulates the uptake and distribution of some basic elements in order to ensure the normal metabolic processes [21]. Cd is transported via cation channels or by other elemental transporters, an increase in the Cd concentration in the soil will lead to ion competition and result in an antagonistic interaction between Cd and other elements in the plant [5, 46]. In the present study, we detected the Zn, Fe, Mn and Cu contents of root and shoot exposed to 10 to 50 μmol/L Cd. Accumulating Cd to different organs of *S. matsudana* had an obvious impact on the uptake of Cu, Fe, Mn, and Zn in the present investigation. The concentration of Mn in roots and shoots was significantly decreased, which agreed with previous studies [20, 21, 22, 26]. In contrast with Mn, the concentrations of Cu, Zn, and Fe in roots *S. matsudana* were significantly increased under Cd stress in this study. D'Alessandro et al. [47] demonstrated that Cd shares chemical similarity to Zn, Fe, and Mn and may compete with them for translocation in plants. Therefore, Cd can selectively share different micronutrient uptake and translocation pathway in plants, and further affect the plant growth [26]. Data from the present investigation also revealed that the concentrations of Zn and Fe in shoots of *S. matsudana* treated with all the Cd concentrations, and the shoots of Cu at 50 μmol/L Cd decreased significantly. The trend may be explained by the fact that Cd accumulates mainly in roots, only small amounts translocate to shoots. Therefore, the low Cd level in shoot may not cause significant affection to Zn, Fe and Cu [26]. As indicated by Stravin-skienė and Račaitė [24], the addition of exogenous Ca contributes to an enhancement of the essential mineral elements uptake and these elements are beneficial to plant growth and development. Data from the present investigation also indicated that exogenous Ca could promote the absorption of Cu, Fe, Zn and Mn in roots and shoots of *S. matsudana* to different extent, suggesting that Ca has alleviating effects on the toxicity of Cd. The information available in the present investigation is an important step towards obtaining a better understanding of Ca on the alleviation of Cd toxicity in plants and its effects on other minerals.

Exogenous Ca reduced Cd uptake mainly by reducing its root adsorption and changing its transport mode. In soil, Ca can reduce the Cd absorption of plants. On the one hand, because Ca and Cd have similar chemical properties, Ca is the main competitor of Cd adsorption sites in soil. When Ca and Cd coexist in the adsorption system, Ca can reduce soil to Cd adsorption, thereby reducing the toxic effects of Cd on plants. On the other hand, glutamate is a wound signal in plants, ion channels of the Glutamate receptor-like family act as sensors that convert this signal into an increase in intracellular Ca ion concentration that propagates to distant organs, where defense responses are then induced [48]. Ca ion plays an important role as the second messenger, which can couple extracellular signals with intracellular physiological and biochemical reactions, enhancing plant resistance to environmental stress by stabilizing cell wall, cell membrane structure and inducing the expression of a series of specific genes. Plants sense local signals, and transmit this information throughout the plant body to rapidly activate defense responses in undamaged parts. Therefore, exogenous

Ca increased the intracellular Ca ion concentration of *S. matsudana*, thereby reducing the toxic effects of Cd on *S. matsudana*.

Conclusions

Based on the information provided in this article, it is concluded that:

1. Cd could inhibit the growth of *S. matsudana* seedling, and 5 mmol/L Ca had an alleviating toxic effects on seedlings' growth.
2. Exogenous Ca (5 mmol/L) could decrease the Cd levels significantly ($P<0.05$) in different organs of *S. matsudana* exposed to all Cd concentrations used during the whole experiment, revealing that Ca can be used to alleviate the harmful effect of Cd.
3. The concentrations of Cu, Zn, and Fe in roots *S. matsudana* were significantly increased under Cd stress, and the concentration of Mn in roots and shoots was significantly decreased by Cd stress.
4. Exogenous Ca could promote the absorption of Cu, Fe, Zn and Mn in roots and shoots of *S. matsudana* to different extent, suggesting that Ca has alleviating effects on the toxicity of Cd.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. EL-BELTAGI H.S., MOHAMED H.I. Alleviation of Cadmium Toxicity in *Pisum sativum* L. Seedlings by Calcium Chloride. Not. Bot. Horti. Agrobo. **41**, 157, **2013**.
2. BI X.Y., FENG X.B., YANG Y.G., LI X.D., SHIN G.P.Y., LI F.L., QIU G.L., LI G.H., LIU T.Z., FU Z.Y. Allocation and Source Attribution of Lead and Cadmium in Maize (*Zea Mays* L.) Impacted by Smelting Emissions. Environ. Pollut. **157**, 834, **2009**.
3. RIZWAN M., MEUNIER J.D., DAVIDIAN J.C., POKROVSKY O.S., BOVET N., KELLER C. Silicon alleviates Cd stress of wheat seedlings (*Triticum turgidum* L. cv. Claudio) grown in hydroponics. Environ. Sci. Pollut. R. **23**, 1414, **2016**.
4. HÉDIJI H., DJEBALI W., BELKADHI A., CABASSON C., MOING A., ROLIN D., BROUQUISSE R., GALLUSCI P., CHAIBI W. Impact of long-term cadmium exposure on mineral content of *Solanum lycopersicum* plants: consequences on fruit production. S. Afr. J. Bot. **97**, 176, **2015**.
5. ZHANG X.F., ZHANG X.H., GAO B., LI Z.A., XIA H.P., LI H.F., LI J. Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of an energy crop, king grass (*Pennisetum americanum* × *P. purpureum*). Biomass Bioenerg. **67**, 179, **2014**.
6. TOMAR P.C., LAKRA N., MISHRA S.N. Effect of cadaverine on *Brassica juncea* (L.) under multiple stress. Indian. J. Exp. Biol. **51**, 758, **2013**.
7. WU H.F., WANG J.Y., LI B.B., OU Y.J., JIANG W.S., LIU D.H., ZOU J.H. Uptake and Accumulation of Cadmium and Relative Gene Expression in Roots of Cd-resistant *Salix matsudana* Koidz. Pol. J. Environ. Stud. **25**, 2717, **2016**.
8. SHI Q.Y., WANG J.R., ZOU J.H., JIANG Z., WANG J.Y., WU H.F., JIANG W.S., LIU D.H. Cadmium uptake and accumulation and its toxic effects on leaves in *Hordeum vulgare*. Fresen. Environ. Bull.**24**, 4504, **2015**.
9. SHI Q.Y., WANG J.R., ZOU J.H., JIANG Z., WANG J.Y., WU H.F., JIANG W.S., LIU D.H. Cd subcellular localization in root tips of *Hordeum vulgare*. Pol. J. Environ. Stud. **25**, 903, **2016**.
10. DENG G., LI M., LI H., YIN L.Y., LI W. Exposure to cadmium causes declines in growth and photosynthesis in the endangered aquatic fern (*Ceratopteris pteridoides*). Aquat. Bot. **112**, 23, **2014**.
11. OSMOLOVSKAYA N.G., DUNG V.V., KUDRYASHOVA Z.K., KUCHAEVA L.N., POPOVA N.F. Effect of Cadmium on Distribution of Potassium, Calcium, Magnesium, and Oxalate Accumulation in *Amaranthus cruentus* L. Plants. Russ. J. Plant Physl. **65**, 553, **2018**.
12. FERROL N., TAMAYO E., VARGAS P. The heavy metal paradox in *arbuscular mycorrhizas*: from mechanisms to biotechnological applications. J. Exp. Bot. **67**, 6253, **2016**.
13. CHOPPALA G., SAIFULLAH, BOLAN N., BIBI S., IQBAL M., RENGEL Z., KUNHIKRISHNAN A., ASHWATH N., OK Y.S. Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. Crit. Rev. Plant Sci. **33**, 374, **2014**.
14. CHEVAL C., ALDON D., GALAUD J.P., RANTY B. Calcium/calmodulin-mediated regulation of plant immunity. BBA-Mol. Cell Res. **1833**, 1766, **2013**.
15. POOVAIAH B.W., DU L.Q., WANG H.Z., YANG T.B. Recent Advances in Calcium/Calmodulin-Mediated Signaling with an Emphasis on Plant-Microbe Interactions. Plant Physiol.**163**, 531, **2013**.
16. CHEONG Y.H., SUNG S.J., KIM B.G., PANDEY G.K., CHO J.S.,.KIM K.N., LUAN S. Constitutive overexpression of the calcium sensor CBL5 confers osmotic or drought stress tolerance in *Arabidopsis*. Mol. Cells. **29**, 159, **2010**.
17. YANG J.L., CHEN Z., WU S.Q., CUI Y., ZHANG L., DONG H., YANG C.P., LI. C.H. Overexpression of the *Tamarix Hispida* ThMT3 Gene Increases Copper Tolerance and Adventitious Root Induction in *S alix Matsudana* Koidz. Plant Cell Tiss. Org. **121**, 469, **2015**.
18. YANG J.X., LI X.L., HU Y.B., GAO L.M., YAO D.X. Enrichment Characteristics of heavy metal cadmium in woody plants system. Kem. Ind. **64**, 283, **2015**.

19. ZOU J.H., WANG G., JI J., WANG J.Y., WU H.F., OU Y.J., LI B.B. Transcriptional, physiological and cytological analysis validated the roles of some key genes linked Cd stress in *Salix matsudana* Koidz. Environ. Exp. Bot. **134**, 116, **2017**.
20. WU H.F., WANG J.Y., LI B.B., OU Y.J., WANG J.R., SHI Q.Y., JIANG W.S., LIU D.H., ZOU J.H. *Salix matsudana* Koidz Tolerance Mechanisms to Cadmium: Uptake and Accumulation, Subcellular Distribution, and Chemical Forms. Pol. J. Environ. Stud. **25**, 1739, **2016**.
21. ZOU J.H., SHANG X.S. LI C.H., OU Y.J., LI B.B., Liu X.J. Effects of Cadmium on Mineral Metabolism and Antioxidant Enzyme Activities in *Salix matsudana* Koidz. Pol. J. Environ. Stud. **28**, 989, **2019**.
22. SUN S., LI M., ZOU J.H., JIANG W.S., LIU D.H. Cadmium effects on mineral accumulation, antioxidant defence system and gas exchange in *cucumber*. Zemdirbyste.**102**, 193, **2015**.
23. ZENG L.H., ZHU T., GAO Y., WANG Y.T., NING C.J., BJÖRN L.O., CHEN D., LI S.H. Effects of Ca addition on the uptake, translocation, and distribution of Cd in *Arabidopsis thaliana*. Ecotox. Environ. Safe. **139**, 228, **2017**.
24. STRAVINSKIENĖ V., RAČAITĖ M. Impact of cadmium and zinc on the growth of white clover (*Trifolium repens* L.) shoots and roots. Pol. J. Environ. Stud.**23**, 1355, **2014**.
25. OUYANG J., LI B.B., LI C.H., SHANG X.S., ZOU J.H. Cadmium Effects on Mineral Accumulation and Selected Physiological and Biochemical Characters of *Salix babylonica* L. Pol. J. Environ. Stud.**26**, 2667, **2017**.
26. LI N.N., LI S.T., WANG S.F., XIE D.T., LUO F. How exogenous cadmium affects micronutrients accumulation and the related gene expression regulation in *Brassica juncea*. Int. J. Agric. Biol. **20**, 2074, **2018**.
27. XU Q.S., MIN H.L., CAI S.J., FU Y.Y., SHA S., XIE K.B., DU K.H. Subcellular distribution and toxicity of cadmium in *Potamogeton crispus* L. Chemosphere. **89**, 114, **2012**.
28. HUANG D.L., GONG X.M., LIU Y.G., ZENG G.M., LAI C., BASHIR H., ZHOU L., WANG D.F., XU P., CHENG M., WAN J. Effects of calcium at toxic concentrations of cadmium in plants. Planta. **245**, 863, **2017**.
29. AHMAD P., BHARDWAJ R., TUTEJA N. Plant signaling under abiotic stress environment. In: P. Ahmad and M.N.V. Prasad (eds.), Adaptations and Stress Tolerance of Plants in the Era of Climate Change. Springer Science+Business Media, LLC, 297, **2012**
30. SARWAT M., AHMAD P., NABI G., HU X.Y. Ca^{2+} signals: The versatile decoders of environmental cues. Crit. Rev. Biotechnol. **33**, 97, **2013**.
31. ANDOSCH A., AFFENZELLER M.J., LÜTZ, C., LÜTZ-MEINDL U. A freshwater green alga under cadmium stress: ameliorating calcium effects on ultrastructure and photosynthesis in the unicellular model *Micrasterias*. J. Plant Physiol. **169**, 1489, **2012**.
32. LÓPEZ-CLIMENT M.F., ARBONA V., PÉREZ-CLEMENTE R.M., ZANDALINAS S.I., GÓMEZ-CADENAS A. Effect of cadmium and calcium treatments on phytochelatin and glutathione levels in citrus plants. Plant Biology. **16**, 79, **2014**.
33. LI P., ZHAO C.Z., ZHANG Y.Q., WANG X.M., WANG X.Y., WANG J.F., WANG F., BI Y.R. Calcium alleviates cadmium-induced inhibition on root growth by maintaining auxin homeostasis in *Arabidopsis seedlings*. Protoplasma. **253**, 185, **2016**.
34. HAYAT S., AHMAD A., WANI A.S., ALYEMENI M.N., AHMAD A. Regulation of Growth and Photosynthetic Parameters by Salicylic Acid and Calcium in *Brassica juncea* under Cadmium Stress. Z. Naturforsch. C. **69**, 452, **2014**.
35. EL-BEITAGI H.S., MOHAMED H.I. Alleviation of Cadmium Toxicity in *Pisum sativum* L. Seedlings by Calcium Chloride. Not. Bot. Horti. Agrobo. **41**, 157, **2013**.
36. NELSON M.T. Interactions of divalent cations with single calcium channels from rat brain synaptosomes. J. Gen. Physiol. **87**, 201, **1986**.
37. JIANG X.J., LUO Y.M., LUO Q., LIU S.L., ZHAO Q.G. Effect of cadmium onnutrient uptake and translocation by Indian Mustard. Environ. Geochem. Hlth. **26**, 319, **2004**.
38. BRUNE A., DIETZ K.J. A comparative analysis of element composition of roots and leaves of barley seedlings grown in the presence of toxic cadmium, molybdenum, nickel, and zinc concentrations. J. Plant Nutr. **18**, 853, **1995**.
39. HAYAKAWA N.,TOMIOKA R.,TAKENAKA C. Effects of calcium on cadmium uptake and transport in the tree species Gamblea innovans. Soil Sci. Plant Nutr. **557**, 691, **2011**.
40. ELLER F., BRIX H. Influence of low calcium availability on cadmium uptake and translocation in a fast-growing shrub and a metal-accumulating herb. Aob. Plants. **8**, **2016**.
41. GONG X.M., LIU Y.G., HUANG D.L., ZENG G.M., LIU S.B., TANG H., ZHOU L., HU X., ZHOU Y.Y., TAN X.F. Effects of exogenous calcium and spermidine on cadmium stress moderation and metal accumulation in *Boehmeria nivea* (L.) Gaudich. Environ. Sci. Pollut. Res. **23**, 8699, **2016**.
42. SCHREIBER, L., HARTMANN, K., SKRABS, M., ZEIER, J. Apoplastic barriers in roots: chemical composition of endodermal and hypodermal cell walls. J. Exp. Bot. **50**, 1267, **1999**.
43. BAXTER I., HOSMANI P.S., RUS A., LAHNER B., BOREVITZ J.O., MUTHUKUMAR B., MICKELBART M.V., SCHREIBER L., FRANKE R.B., SALT D.E. Root suberin forms an extracellular barrier that affects water relations and mineral nutrition in *Arabidopsis*. Plos Genet. **5**, 188, **2009**.
44. HANSCH R., MENDEL R.R. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Curr. Opin. Plant Biol. **12**, 259, **2009**.
45. LIU J., LI K.Q., XU J.K., LIANG J.S., LU X.L., YANG J.C., YANG J.C., ZHU Q.S. Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotypes. Field Crop. Res. **83**, 271, **2003**.
46. CLEMENS S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie. **88**, 1707, **2006**.
47. D'ALESSANDRO A., TAAMALLI M., GEVI F., TIMPERIO A.M., L. ZOLLA L., GHNAYA T. Cadmium stress responses in *Brassica juncea*: hints from proteomics and metabolomics. J. Proteome Res. **12**, 4979, **2013**.
48. TOYOTA M., SPENCER D., SAWAI-TOYOTA S., WANG J.Q., ZHANG T., KOO A.J., HOWE G.A., GILROY S. Glutamate triggers long-distance, calcium-based plant defense signaling. Science. **361**, 1112, **2018**.