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Phytotoxicity of Trace Elements on Root Elongation and Hypocotyl Growth in *Arabidopsis thaliana*

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We investigated phytotoxicities of 18 elements (Li, B, Na, Ca, V, Mn, Fe, Co, Ni, Cu, Mo, Zn, Rb, Sr, Sn, Cs, Ba and Pb) to *Arabidopsis thaliana*. Toxicity was quantified as an effective concentration value (EC_{50}) which showed 50% decrease of root and hypocotyl length. Ranges of the EC_{50} value for root elongation were the following: <0.1 mM (V and Sn), 0.1-1 mM (Fe, Co, Ni, Cu, Mo and Pb), 1-10 mM (B, Mn, Zn, Cs and Ba), and 10-100 mM (Li, Na, Ca, Rb and Sr). Those for hypocotyl growth were 0.1-1 mM (V, Fe, Co, Ni, Cu, Mo and Sn), 1-10 mM (B, Mn, Zn, Cs and Ba), and 10-100 mM (Li, Na, Ca, Rb and Sr). In the cases of B, V, Co, Ni, Sn, Ba and Pb, the EC_{50} values for root elongation were lower than those for hypocotyl growth, suggesting that root elongation was more sensitive to those elements compared with hypocotyl growth. On the other hand, hypocotyl growth was more sensitive to Na, Rb and Cs compared with root elongation. The root elongation and hypocotyl growth were similarly sensitive to Li, Ca, Fe, Mo and Zn.

Keywords: *Arabidopsis*; hypocotyl growth; phytotoxicity; root elongation; trace element

I. INTRODUCTION

Acquisition of mineral nutrition by plants is crucial for plant growth and subsequently for all living organisms including humans. Plants show optimal growth under adequate concentrations of nutrients in the tissue. When the nutrient concentrations are too low or high, plants exhibit deficiency or toxicity symptoms (Marschner, 1995). In order to avoid damage of crops by depletion or excess of nutrients, it is important to develop a technology to improve productivity. Recently, acquisition of heavy metals by plants is also investigated in the context of phytoremediation (Ali *et. al.*, 2013; Nagajyoti *et. al.*, 2010; Meagher, 2000; Salt *et al.*, 1995). Phytoremediation is a technology to remove harmful heavy metals from contaminated soil with the use of plants and is expected to be a solution to the environmental pollution problems. Effects of these mineral nutrients and heavy metals on plants were investigated for many plant species such as agricultural crops species and cultivars growing on various soils (Ajasa *et. al.*, 2004; Baghour *et. al.*, 2001; Khan *et. al.*, 2015; Marchiol *et. al.*, 2004; Munzuroglu & Geckil, 2002; Nazar *et. al.*, 2012; Rizwan *et. al.*, 2016; Vernay *et al.*, 2007). However, the effects of a number of non-essential elements have not been reported. In order to discuss the effect of various elements including non-essential elements on the plants, researchers attempt to generate and screen for those element-resistant plants by biotechnology. At the beginning of generating element-resistant plants, we have to know the nutritional character of wild-type plants against the target elements.

We report here the results of the phytotoxic effects of 18 elements, including the essential nutrients and harmful heavy metals (Ca, Mo, Cu, Zn, Mn, Fe, B, Ni, Sn and Pb), and beneficial elements and the other elements (Na, Co, Li,

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Rb, Cs, Sr, Ba and V), on *Arabidopsis thaliana* root and hypocotyl length.

II. MATERIALS AND METHODS

A. Chemicals

Murashige and Skoog salts and Gelrite® were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan and Merck and Co., Inc., Rahway, NJ, USA, respectively. All reagents of metal salts (>99% grade), LiCl, NaCl, RbCl, CsCl, $Ca(NO_3)_2$, $SrCl_2{\cdot}6H_2O$, $BaCl_2{\cdot}2H_2O$, VCl_3, $MnSO_4$ $5H_2O$, $FeSO_4{\cdot}7H_2O$, $CoCl_2{\cdot}6H_2O$, $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}7H_2O$, $PbCl_2$, and $SnCl_2{\cdot}2H_2O$, were obtained from Kanto Chemical Co. Inc., Tokyo, Japan except for H_3BO_3, $NiCl_2{\cdot}6H_2O$ and $Na_2MoO_4{\cdot}2H_2O$ (Wako Pure Chemical Industries). Pure water was used for preparing all solutions.

B. Plant Materials and Growth Conditions

Seeds of *Arabidopsis thaliana* ecotype Columbia Col-0 were surface sterilised with a mixture of 2% NaClO and 0.003% Triton X-100 for 6 min and washed for five times with sterile water. The seeds were resuspended in sterile 0.1% (w/v) Gelrite® agar. The basal solid medium contained Murashige and Skoog (MS) salts (Murashige & Skoog, 1962) with 0.1% (w/v) sucrose and 0.38% (w/v) Gelrite®. The pH of all media was adjusted to 5.5 prior to autoclave sterilisation. The MS medium consisted of the following salts: 20.6 mM NH_4NO_3, 18.8 mM KNO_3, 3.0 mM $CaCl_2{\cdot}2H_2O$, 1.5 mM $MgSO_4{\cdot}7H_2O$, 1.3 mM KH_2PO_4, 0.1 mM H_3BO_3, 0.1 mM $FeSO_4{\cdot}7H_2O$, 0.1 mM $MnSO_4{\cdot}4H_2O$, 0.03 mM $ZnSO_4{\cdot}7H_2O$, 0.005 mM KI, 0.001 mM $Na_2MoO_4{\cdot}2H_2O$, 0.0001 mM $CuSO_4{\cdot}5H_2O$, 0.0001 mM $CoCl_2{\cdot}6H_2O$, and 0.1 mM ethylenediaminetetraacetic acid disodium salt. The modified MS agar plates used in this work were prepared by addition of each element at the following range of concentration to the molten basal medium: 0.003-0.3 mM of V or Cu; 0.01-1 mM of Co, Ni, Sn or Pb; 0.03-3 mM of Zn or Mo; 0.1-10 mM of B, Mn, Fe or Cs; 0.1-100 mM of Li, or Rb; 0.3-10 mM of Ba; 3-100 mM of Ca; 3-300 mM of Na. Twenty seeds were sown in rows onto each plate (120 × 120 × 17 mm). The plates were placed vertically in a growth chamber maintained at 23 ± 2˚C. Light provided by cool-white fluorescent bulbs was at 84.5 ± 2.5 µmol m^{-2} s^{-1} with a daily cycle of 8 h light and 16 h dark. The plates were sealed to maintain internal humidity. Lengths of root and hypocotyl in 12-day-old (after sowing) seedlings were measured using a ruler, and the mean of 10 measurements was used for analysis.

III. RESULTS AND DISCUSSION

A. Effect of Elements on Root Elongation and Hypocotyl Growth

The results of root elongation on plates containing various concentrations of each of the 18 elements were shown in Figures 1-3, and those of hypocotyl growth were shown in Figures 4-6. In all cases, as the element concentration in media increased, both the root and hypocotyl length decreased. The seeds sown onto MS medium containing elements at or above the following concentrations did not germinate after 10 d: 0.3 mM Cu; 1 mM Fe, Ni and Sn; 3 mM Zn and Mo; 10 mM B; 30 mM Li; 100 mM Ca; and 300 mM Na. The MS medium containing 100 mM Sr did not solidify, and the effect of Sr over 30 mM was not shown in Figures 1 and 4 because of the deficiencies of MS solid medium.

B. Alkaline Metal and Alkaline Earth Metal Elements

With the exception of Cs, three alkaline metals, Li, Na and Rb, did not indicate inhibitory effect on root elongation up to 3 mM, and the root elongation only began to decrease over 3 mM. However, in the case of Cs, root elongation began to decrease over 1 mM (Figure 1). Zhu *et al.* (1998) reported the same tendency for root elongation inhibition by Cs to occur below 3 mM, but inhibition by Li and Na only began above 3 mM. With reference to the relative root elongation and hypocotyl growth, each represented by the ratio of root and hypocotyl length of *A. thaliana* growing on modified MS plates containing indicated concentrations of each element to that growing on the basal MS Gelrite® plate, hypocotyl growth was more sensitive to alkaline metals compared to root elongation (Figures 1 & 4). Although relative root elongation and hypocotyl growth were decreased to 60% at 3 mM Li, Na and Rb, those were decreased to about 20% at 3 mM Cs. Therefore, Cs was the most toxic element for *A. thaliana* of the four alkaline metals tested in this study.

Cs is a member of the alkaline metal group as well as K that is an essential macronutrient, it is also an inhibitor of K^+ channels (Baizabal-Aguirre *et al.*, 1999; Becker *et al.*, 1996; Fan *et al.*, 1999). K has a number of physiological roles in plants, such as enzyme activation, photosynthesis, cell extension and osmoregulation. The inhibition of K^+ channel by Cs was indirectly indicated by comparing the concentration of Cs that was used as general inhibitor of K^+ channel *in vitro* and that supplemented as an additional element into culture medium *in vivo*. The concentration (1-10 mM Cs) that indicated remarkable inhibition of root elongation and hypocotyl growth *in vivo*

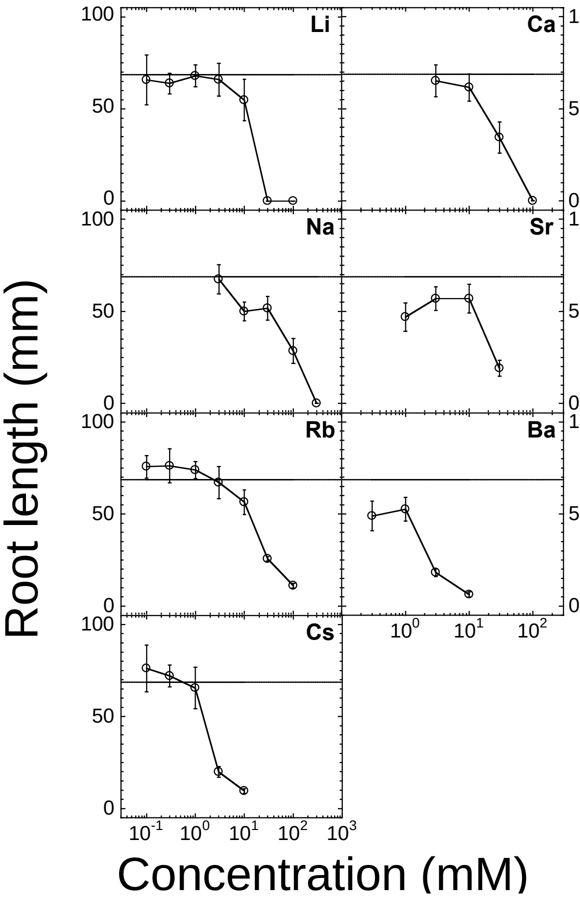

Figure 1. Root elongation in the presence of varying concentrations of alkaline and alkaline earth metals. The control root elongation was 67.2 ± 1.7 mm (shown as horizontal lines). Data were shown as the mean ± SD of 10 measurements.

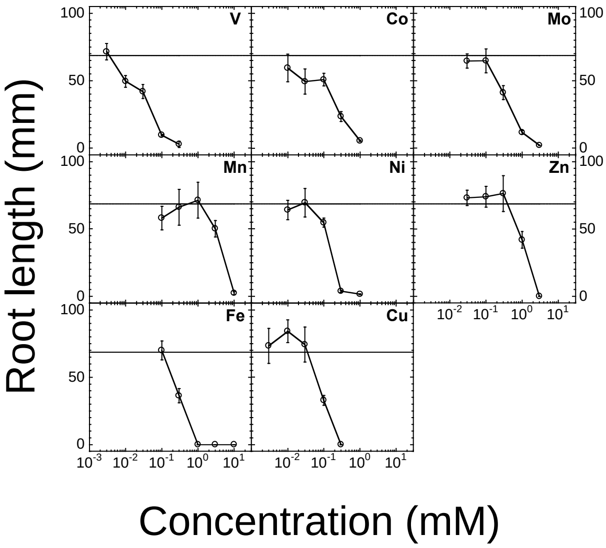

Figure 2. Root elongation in the presence of varying concentrations of transition metals. The control root elongation was 67.2 ± 1.7 mm (shown as horizontal lines). Data were shown as the mean ± SD of 10 measurements.

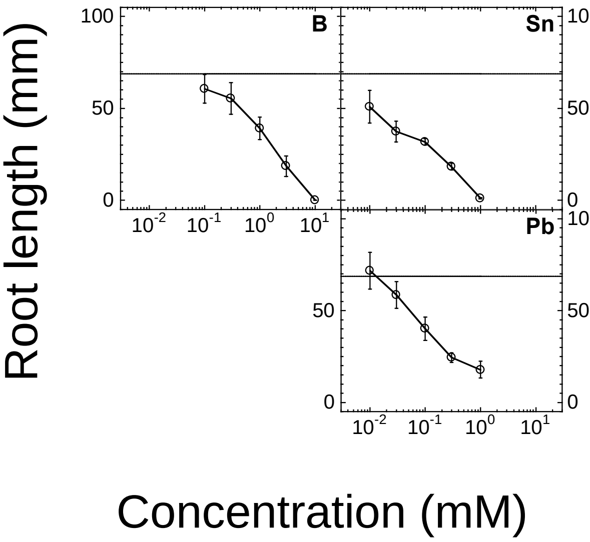

Figure 3. Root elongation in the presence of varying concentrations of B, Sn and Pb. The control root elongation was 67.2 ± 1.7 mm (shown as horizontal lines). Data were shown as the mean ± SD of 10 measurements.

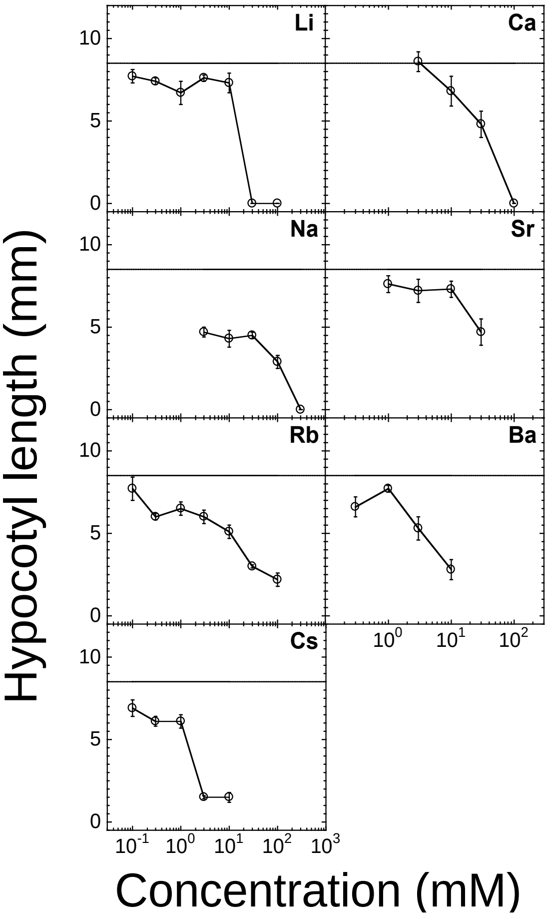

Figure 4. Hypocotyl growth in the presence of varying concentrations of alkaline and alkaline earth metals. The control hypocotyl growth was 8.5 ± 0.1 mm (shown as horizontal lines). Data were shown as the mean ± SD of 10 measurements.

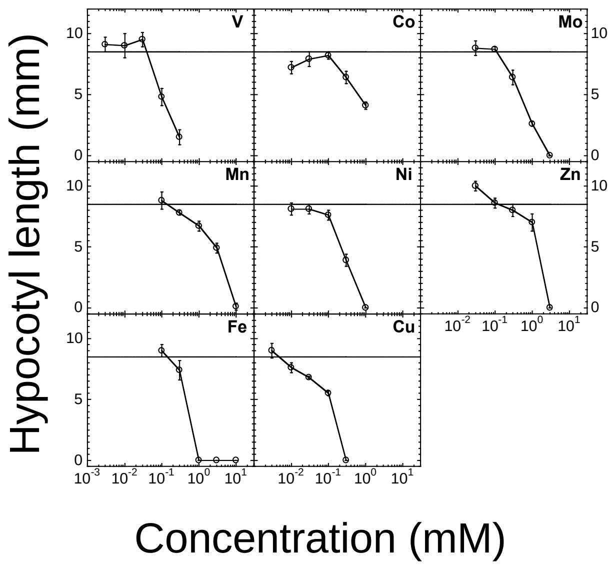

Figure 5. Hypocotyl growth in the presence of varying concentrations of transition metals. The control hypocotyl growth was 8.5 ± 0.1 mm (shown as horizontal lines). Data were shown as the mean ± SD of 10 measurements.

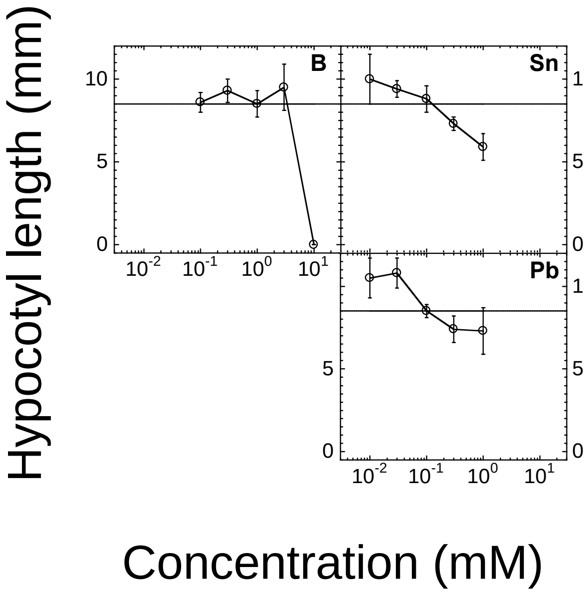

Figure 6. Hypocotyl growth in the presence of varying concentrations of B, Sn, and Pb. The control hypocotyl growth was 8.5 ± 0.1 mm (shown as horizontal lines). Data were shown as the mean ± SD of 10 measurements.

in this study was close to the concentration (3 mM Cs) that inhibited about 95% of K^+ transport activity of KAT1 and AKT2 (members of K^+ channels in *A. thaliana*) *in vitro* (Baizabal-Aguirre *et al.*, 1999). As such, Cs was considered as the most toxic of the four alkaline metals (Li, Na, Rb and Cs) in plant.

The effects of alkaline earth metal elements on root elongation and hypocotyl growth were also investigated. There was a tendency for the extent of growth inhibition to increase as the atomic number increased (Figures 1 & 4). Of the three alkaline earth metal elements tested in this study, Ba was the most toxic element for *A. thaliana*.

C. Transition Metal Elements

Inhibition of relative growth by increasing transition metals concentration in medium showed the same tendency between roots and hypocotyls with the exception of V and Co (Figures 2 & 5). Root elongation was more sensitive to V and Co than hypocotyl growth. Hypocotyl growth was not influenced by V up to 0.03 mM and by Co up to 0.1 mM, but root elongation decreased over 0.003 mM of V and 0.01 mM of Co, respectively. The concentrations that indicated remarkable inhibition of root elongation were the following: 0.3 mM V, Ni and Cu;

1 mM Fe and Co; 3 mM Zn and Mo; and 10 mM Mn. Concentrations of transition metal elements inhibiting hypocotyl growth were as follows: 0.3 mM V and Cu; 1 mM Fe and Ni; 3 mM Zn and Mo; and 10 mM Mn.

Tu and Sliwinski (1985) reported that vanadate inhibited the activity of ATPase expressed in root plasma membrane in *Zea mays*. We could not say for certain whether chemical form of added VCl_3 existed in MS Gelrite® medium as vanadate in this study. However, the value of EC_{50} of V for root was calculated to be 40 µM from Figure 2 (Table 1) and this value was close to the inhibition constant of ATPase against vanadate at 58 µM (Szafran & Haaker, 1995). For the reasons mentioned above, one explanation for strong inhibition of root elongation by V may be that V was effective in inhibiting root elongation. It is possible that inhibition of hypocotyl growth by V was caused as a secondary effect of inhibition of root elongation.

D. Other Elements

Root elongation was more sensitive to B, Sn and Pb compared to hypocotyl growth (Figures 3 & 6). Hypocotyl growth was almost completely inhibited by B and Sn in excess concentrations. Increasing Pb concentration in medium inhibited root elongation, but the extent of hypocotyl growth inhibition was less than that of root elongation. Excess concentration of B inhibited root elongation according to increase in B concentration, but hypocotyl growth was not affected by B at a concentration less than or equal to 10 mM.

In the case of Sn, and Pb, in particular, inhibition of root elongation occurred at concentration one order of magnitude lower than that found to inhibit hypocotyl growth. The element distribution ratio of these metals between roots and shoots of *A. thaliana* that was cultivated by hydroponics with addition of those metals in medium indicated that about 95% of the two metals were distributed in root parts (data not shown). These results were in agreement with Qureshi *et al.* (1986) who described that very little Pb was translocated from root part to above ground part and that Pb precipitated out of solution as a phosphate complex in root cell wall. It was considered that inhibition of hypocotyl growth by Pb was due to the resultant root elongation inhibition by that metal.

Table 1. Effective concentration of various trace elements on root elongation and hypocotyl growth of *Arabidopsis thaliana*

Element	**EC_{50} value (mM)**	
	Root	**Hypocotyl**
Li	15	16
Na	75	36
Rb	22	16
Cs	2.1	1.5
Ca	31	34
Sr	20	30
Ba	1	5
V	0.04	0.3
Mn	4.5	3.5
Fe	0.32	0.5
Co	0.2	0.9
Ni	0.16	0.26
Cu	0.1	0.13
Zn	1.2	1.6
Mo	0.4	0.6
B	1.3	6
Sn	0.06	0.5
Pb	0.16	>1.0

The EC50 value or concentration of elements that caused 50% inhibition of root elongation and hypocotyl growth was determined from data used to construct Figures 1–6.

E. Effective Concentration for Root and Hypocotyl Growth Inhibition

To compare the inhibitory effects of various elements on root elongation and hypocotyl growth, the effective concentration of elements that caused 50% inhibition of root elongation and hypocotyl growth of the plants (EC_{50}) was determined from Figures 1-6 and summarised in Table 1. Based on the EC_{50} values for root growth inhibition, the elements were arranged in an order of decreasing inhibition efficiencies: V > Sn > Cu > Ni, Pb > Co > Fe > Mo > Zn ≥ B ≥ Ba ≥ Cs > Mn > Li > Sr ≥ Rb > Ca > Na. Judging from the EC_{50} values for root elongation inhibition of *A. thaliana*, V was the most and Na the least toxic among these elements. Similarly, based on these values for hypocotyl growth inhibition, except for Pb for which the EC_{50} value was not determined, the elements were arranged in an order of decreasing inhibition efficiencies: Cu > Ni ≥ V ≥ Fe, Sn ≥ Mo > Co > Cs, Zn > Mn > Ba ≥ B > Rb, Li > Sr ≥ Ca ≥ Na. Judging from the

EC_{50} values for hypocotyl growth inhibition of *A. thaliana*, Cu was the most and Na the least toxic of those elements. Comparison of the EC_{50} values for root and hypocotyl growth inhibition showed that root elongation was more sensitive to B, V, Co, Ni, Sn, Ba and Pb compared to hypocotyl growth. On the other hand, hypocotyl growth was more sensitive to Na, Rb and Cs than root elongation. The root elongation and hypocotyl growth were similarly sensitive to Li, Ca, Fe, Mo and Zn. In this study, inhibition of root elongation by Co and Pb, and that of hypocotyl growth by Cu were described in *A. thaliana*. The results were in agreement with a previous report of a similar experiment using another dicot plant, the cucumber (Munzuroglu & Geckel, 2002).

The purpose of this study was to estimate the phytotoxicity of a number of elements on a single plant species, i.e. *A. thaliana*, under the same cultivation condition. This study lays foundation for understanding the role of those elements in plant physiology. Recent progress in genetic engineering made it possible to analyse the physiological mechanisms at molecular and genetic levels in plants. Therefore, we are able to discuss the effect of elements on plants at molecular and genetic levels by constructing mutant plants that are sensitive or insensitive to a specific element. *Arabidopsis* mutants that were sensitive or insensitive to B, Na, Cu, Cs, Al and Mn have contributed to an understanding of the physiological roles of those elements in plants (Bian *et al.*, 2018; Delhaize, 1996; Larsen *et al.*, 1996; 1998; Murphy *et al.*, 1995; Noguchi *et al.*, 1997; Sheahan *et al.*, 1993; Takano *et al.*, 2002; van Vliet *et al.*, 1995; Wu *et al.*, 2015; Zhu *et al.*, 1998).

Our results may provide a fundamental knowledge for studies that screen for mutant plants responding to specific elements. *Arabidopsis thaliana* used in this study is the first higher plant to have its genome sequence analysis completed. Moreover, the plant was not cultivated in specific soil or medium, but MS medium that is widely used. Thus, our results are feasible for application across a number of experimental systems.

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