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The Effect of Ca^{2+} on Cd^{2+}-induced Physiological Toxicity in *Commelina communis* L.

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Abstract - 3-weeks old *Commelina* was transferred to and grown in Hoagland solution (±100 μM Cd^{2+}, 100 μM Cd^{2+}+100 μM Ca^{2+}, 100 μM Cd^{2+}+200 μM EGTA) for two weeks and then a number of physiological activities was investigated. Cd^{2+} reduced total chlorophyll content up to 29% at a week and 75% at two weeks. In the treatment of Cd^{2+}+Ca^{2+}, the total chlorophyll content was reduced to 29% at a week and 80% at two weeks. Cd^{2+} reduced 24% of Fv/Fm after two weeks. In case of Cd^{2+}+Ca^{2+}, Fv/Fm was reduced 55% at a week, but after two weeks, the plants were almost dead and Fv/Fm could not be measured. When EGTA was treated with Cd^{2+}, the value of Fv/Fm was not affected. There were no differences of water potential between the control and the treatment of Cd^{2+}+EGTA for a week, but in other treatments. water potential was reduced. Cd^{2+} reduced about 21% of water potential and Cd^{2+}+Ca^{2+} reduced 43% of water potential after two weeks. Cd^{2+} inhibited 21% of photosynthetic activity at a week and 32% at two weeks. In case of photosynthetic activity, Cd^{2+}+Ca^{2+} inhibited 58% at a week and 73% at two weeks. Cd^{2+}+EGTA inhibited 15% of photosynthetic activity at a week and 21% at two weeks. Similar results were found in stomatal conductance. From the above results, it was observed that the treatment of Ca^{2+} with Cd^{2+} induced more reduction of a series of physiological responses than those of the treatment of Cd^{2+} alone. Therefore, it could be concluded that Ca^{2+} did not reduce the toxicity of Cd^{2+}, but enhanced Cd^{2+}-induced physiological toxicities, but EGTA induced an decrease of Cd^{2+}-induced physiological toxicities.

Key words : cadmium-tocixities, calcium, *Commelina*, EGTA

INTRODUCTION

Trace metal pollution is among the most pervasive and serious environmental problems facing the biosphere. Especially, heavy metals such as Cd, Pb, and Hg exist in aquatic, atmospheric and terrestial environments at low to high concentration depending on natural and man-made disturbances. The degree of pollution by these toxic heavy metals is increasing by industrial and agricultural necessary. The most serious problems by this pollution include firstly, the destruction and change of habitat and secondly, distinction of species leading to the reduction of species diversity.

Fortunately, all organisms contain one or more types of cysteine rich metallothioneins (MTs) which selectively sequester trace metals. Phytochelatins (PCs), typically found in plants are non-translationally synthesized metal-thiolate polypeptides related synthesized metal-thiolate polypeptides and have the repeated sequence; (γ-GluCys)nGly, where n = 2~11 (Maitai *et al.* 1996; Rauser 1995; Steffens 1990).

However, the role of MTs has been limited depending on the exposure time and the concentration of the me-

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tals. It is known that Cd^{2+} is the one of the most harmful heavy metals to human beings and animals (Chaudri *et al.* 1995). When plants were exposed to Cd^{2+}, the reduction of crop yields, defoliation, the inhibition of growth and photosynthetic activity were observed in many plants and the degree of Cd^{2+}-toxicity was proportional to the concentration of Cd^{2+} (Kim 1982; Kim 1992: Kim and Park 1992; Page *et al.* 1972; Willmer 1983).

Ca^{2+} is very important ion and has two values of positive ion like Cd^{2+}. The wall and apoplastic spaces are rich in calcium, but cytosolic Ca^{2+} concentration are maintained at very low levels (in the micromolar range). Small fluctuation in cytosolic Ca^{2+} concentration drastically alter the activities of many enzymes. Cytosolic calcium levels could affect the calcium-activated regulator protein calmodulin. Calmodulin regulates the activity of a number of enzymes such as protein kinases, which is very important protein for a signal transduction. We thought that Ca^{2+} could be the most important candidates for the reduction of Cd^{2+}-toxicity. This means that Ca^{2+} would compete with Cd^{2+} in ion-transport, indicating that Cd^{2+} transport will be reduced. Calcium and calcium-calmodulin complex are second messengers which are involved in the signal transduction. This imply that calcium or calcium-calmodulin complex could be related in the process of PCs synthesis by affecting the genetic expression.

Therefore, in this study the effect of Ca^{2+} on Cd^{2+}-induced physiological toxicities in *Commelina communis* had been investigated.

MATERIALS AND METHODS

Commelina communis L. was grown from seeds in a growth chamber at 22±2°C with supplementary light to give a photoperiod of 13 h. and a photon flux density of 200 μmole m^{-2} s^{-1}. At all stage of development, the plants were kept free from water stress by periodic watering and Phostrogen (plant food: 1 g L^{-1}) was furnished to the plants twice a week.

Three weeks old healthy plants were transferred to Hoagland solution (±100 μM Cd^{2+}, 100 μM Cd^{2+}+100 μM Ca^{2+}, 100 μM Cd^{2+}+200 μM EGTA), into which air was bubbled though hyperdermic needles fitted in the beakers. And they were grown in a growth chamber at the same condition of the plant material for two weeks. The measurements of chlorophyll content (Holden 1965), chlorophyll fluorescence (Fim 1500, ADC), water potential (PMS Instrument Co.), photosynthetic activity and stomatal conductance (L1-6400 Portable Photosynthetic System) were performed (Lee 2000).

RESULTS AND DISCUSSION

Fig. 1 (Lee 2001) shows the effect of Ca^{2+} on the accumulation of Cd^{2+} and the growth of Cd^{2+}-treated *Commelina communis* for a week. In this paper, among the Fig. 1, the data of control and Cd^{2+}+EGTA were not shown. Cd^{2+} significantly reduced the width, length and freshweight of *Commelina* which represent the parameters of plant growth. When Cd^{2+} was treated with Ca^{2+}, the plant growth was more reduced than that of the treatment of Cd^{2+} alone.

Table 1 shows clear differences of chlorophyll content in each treatment. Cd^{2+} reduced total chlorophyll content up to 29% at a week and 75% at two weeks. In the treatment of Cd^{2+}+Ca^{2+}, the total chlorophyll content was reduced to 29% at a week and 80% at two weeks. The reduction of chlorophyll content about 80% represents that the leaves were changed to almost bright yellow and almost dead. In case of Cd^{2+}+EGTA, the total chlorophyll content was reduced to 28% at a week and 54% at two weeks. Accordingly, the treatment of EGTA markedly inhibited the reduction of the chlorophyll content induced by Cd^{2+}. However, the treatment

Table 1. The effects of Ca^{2+} on the change of chlorophyll content (μg/g fr.wt) induced by Cd^{2+} and chl *a/b* ratio in *Commelina communis* L.

Weeks	Chlorophyll	Con	Cd^{2+}	Cd^{2+}+ Ca^{2+}	Cd^{2+}+ EGTA
1	Total chl	740.2	522	522.4	531.9
	Chl *a*	515.3	301	372.3	365.6
	Chl *b*	224.7	220	149.1	166.3
	Chl *a/b*	2.3	1.37	2.5	2.2
2	Total chl	827.9	207	170	377
	Chl *a*	590	126.4	120	278
	Chl *b*	238	80	50	99
	Chl *a/b*	2.5	1.58	2.4	2.81

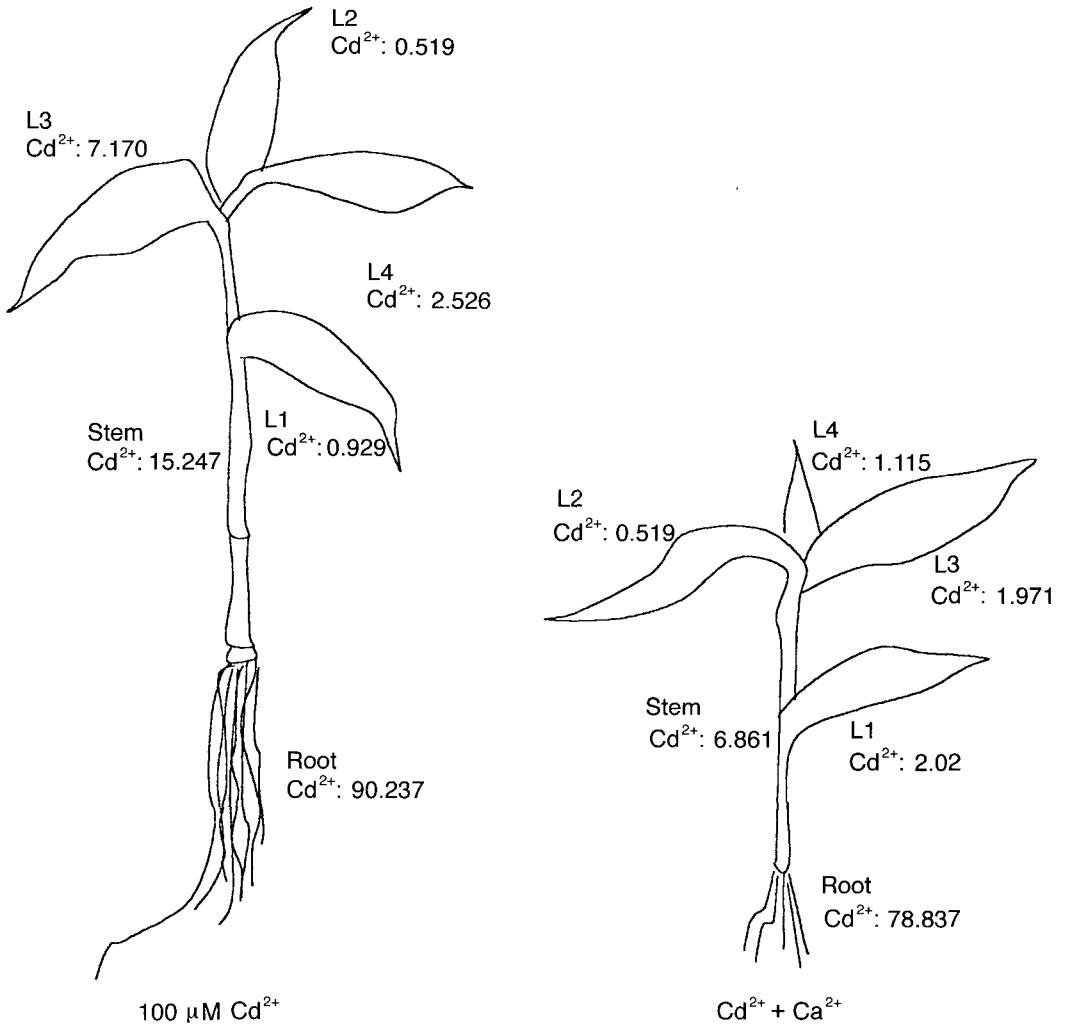

Fig. 1. The effect of Ca^{2+} on the accumulation of Cd^{2+} and growth of *Commelina communis* L. 3-weeks old *Commelina* was transferred to and grown in Hoagland's solution (100 μM Cd^{2+}, 100 μM Cd^{2+}+100 μM Ca^{2+}) for a week. and then the effect of Ca^{2+} on the accumulation of Cd^{2+} and growth of Cd^{2+}-treated *Commelina* was investigated. The unit of the concentration of Cd^{2+} is mg/kg fr.wt. and L represents Leaf. Fig. 1 was selected from Lee (2001) for the understanding of other Figures.

of Ca^{2+} severely stimulated plant damage and enhanced Cd^{2+}-toxicity.

The chlorophyll *a/b* ratio in general belongs to 2.5~3.5 (Oh and Lee 1996). In this study, the range of chlorophyll *a/b* ratio was between 1.4 and 2.8. In control, chlorophyll *a/b* ratio was 2.3~2.5 which belongs to the normal ratio. In contrast, Cd^{2+} treatment changed chlorophyll *a/b* ratio to 1.37~1.58 indicating that chlorophyll *a* was more sensitive to Cd^{2+} than that of chlorophyll *b*. In the treatment of $Cd^{2+}+Ca^{2+}$ and Cd^{2+}+EGTA, total chlorophyll content was greatly decreased, but there was no big difference of chlrophyll *a/b* ratio on contrast to the control. These results suggest that Ca^{2+} could be involved in the stability of chlorophyll *a*. However, it needs more study to understand how Ca^{2+} inhibit the destruction of chlorophyll *a*. The role of EGTA in maintaining of normal chlorophyll *a/b* ratio could be the result of the chelating of Cd^{2+} as same as Ca^{2+} because they commonly have two positive electrical charges.

Fig. 2 shows the effect of Ca^{2+} on the change of chlorophyll fluorescence (Fv/Fm) induced by Cd^{2+} in *Commelina*. The ratio of Fm/Fv is proportional to the activity of photosynthetic reaction center (Demmig and Bjorkman 1987), and particularly is related with photosys-

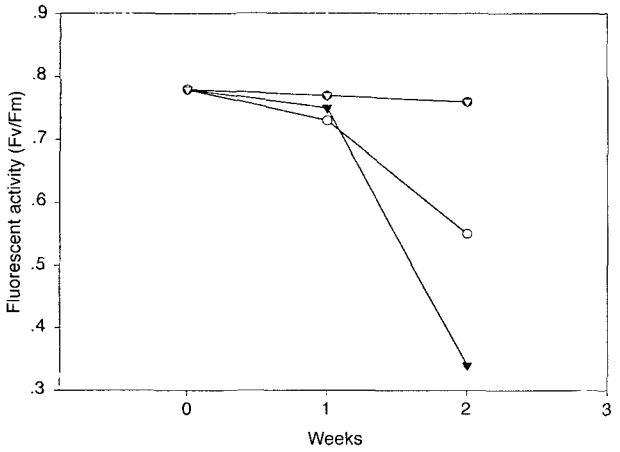

Fig. 2. The effect of Ca^{2+} on the change of chlorophyll fluorescence (Fv/Fm) induced by Cd^{2+} in *Commelina communis* L. Each point is the mean (±s.e.m.) of 2 measurements. Closed circles, Contol; open circle, Cd^{2+}; open triangle, Cd^{2+}+EGTA, closed triangle; $Cd^{2+}+Ca^{2+}$.

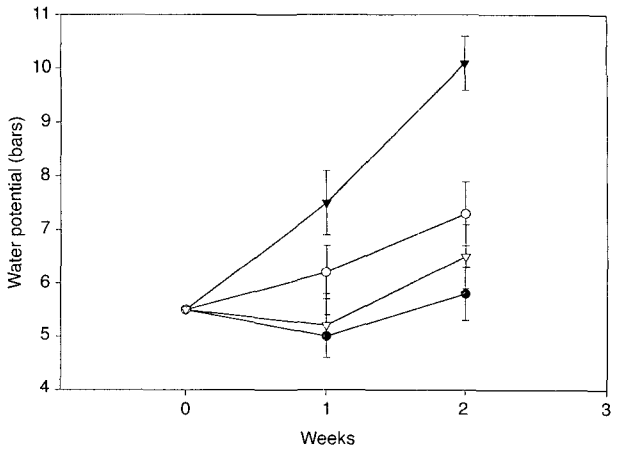

Fig. 3. The effect of Cd^{2+} and Ca^{2+} on water potential of the stem in *Commelina communis* L. Each point is the mean (±s.e.m.) of 2 measurements. Closed circles, Contol; open circle, Cd^{2+}; open triangle, Cd^{2+}+EGTA, closed triangle; $Cd^{2+}+Ca^{2+}$.

tem II (Oquist *et al.* 1992). There were not clear differences in the ratio of Fv/Fm in each treament when the plants were Incubated in Hoagland solution (±100 μM Cd^{2+}, 100 μM Cd^{2+}+200 μM EGTA) for a week, but Cd^{2+} reduced 24% of Fv/Fm after two weeks. In case of $Cd^{2+}+Ca^{2+}$, Fv/Fm was reduced 55% in a week, but after two weeks, the plants were almost dead and Fv/ Fm could not be measured. When EGTA was treated with Cd^{2+}, the value of Fv/Fm was not affected.

Fig. 3 shows the effect of Ca^{2+} on the change of water potential induced by Cd^{2+} in *Commelina*. The concept of water potential has two principal uses. First, the water potential is the quantity that governs the direction of water flow across cell membrane. The second important use of water potential is as a measure of the water status of a plant (Taiz and Zeiger 1991). There were no differences of water potential between the control and the treatment of Cd^{2+}+EGTA for a week, but in other treatments. water potential was reduced. Cd^{2+} reduced about 21% of water potential and $Cd^{2+}+Ca^{2+}$ reduced 43% of water potential after two weeks. In the value of water potential unit (bars), it represents that the lower the value the more water content.

Water deficits lead to inhibition of plant growth and photosynthesis, as well as to other effects (Taiz and Zeiger 1991). The reduction of half percent of water potential observed in the treatment of $Cd^{2+}+Ca^{2+}$ led to the permanent wilting point of the plant indicating that the plant was almost dead.

Fig. 4 and 5 show the measurements of photosynthetic activity and stomatal conductance as a function of light intensity (1,000 μmol m^{-2} s^{-1}) in *Commelina communis* L. Cd^{2+} inhibited 21% of photosynthetic activity at a week and 32% at two weeks. $Cd^{2+}+Ca^{2+}$ inhibited 58% of photosynthetic activity at a week and 73% at two weeks. Cd^{2+}+EGTA inhibited 15% of photosynthetic activity at a week and 21% at two weeks. Similar effect by each treatment was found in terms of stomatal conductance.

Cd^{2+} inhibited 33% of stomatal conductance activity at a week and 58% at two weeks. $Cd^{2+}+Ca^{2+}$ inhibited 78% of stomatal conductance at a week and 85% at two weeks. Cd^{2+}+EGTA inhibited 26% of stomatal conductance at a week and 34% at two weeks.

Stomata opening and closing is related with the photosynthetic activity (Lee and Kim 1997). Therefore, the magnitude of inhibition of photosynthetic activity and stomatal conductance by Cd^{2+} and $Cd^{2+}+Ca^{2+}$ seemed to be similar.

From the above results, it was observed that the treatment of Ca^{2+} with Cd^{2+} was more harmful in series of physiological responses than those of the treatment of Cd^{2+} alone. Therefore, it could be concluded that Ca^{2+}

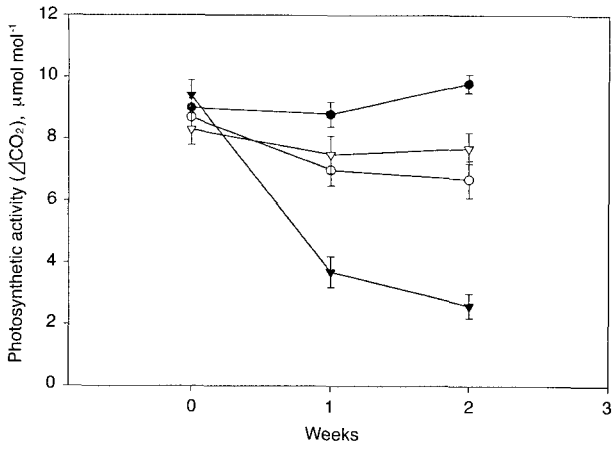

Fig. 4. Photosynthetic activity as a function of light intensity (1,000 μmol m^{-2} s^{-1}) in *Commelina communis* L. Each point is the mean (±s.e.m.) of 2 measurements. Closed circles, Contol; open circle, Cd^{2+}; open triangle, Cd^{2+} + EGTA, closed triangle; Cd^{2+} +Ca^{2+}.

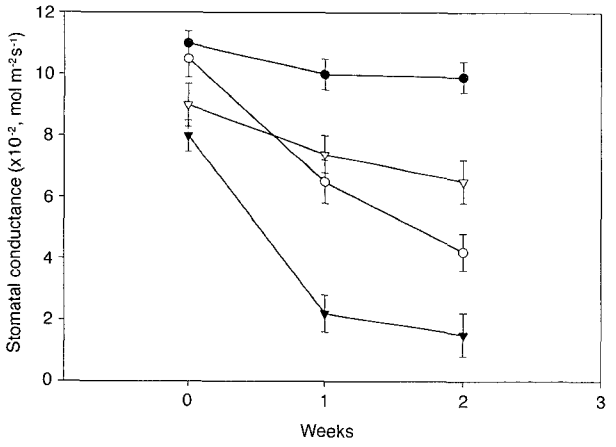

Fig. 5. Stomatal conductance as a function of light intensity (1000 μmol m^{-2} s^{-1}) in *Commelina communis* L. Each point is the mean (±s.e.m.) of 2 measurements. Closed circles, Contol; open circle, Cd^{2+}; open triangle, Cd^{2+}+EGTA, closed triangle; Cd^{2+} +Ca^{2+}.

did not reduce the toxicity of Cd^{2+}, but enhanced Cd^{2+}-induced toxicities and EGTA induced an decrease of Cd^{2+}-induced toxicities. These phenomena came from the results that Ca^{2+} inhibited Cd^{2+} transport into the plant, but Ca^{2+} itself absorbed into the plant and induced Ca^{2+}-toxicity. In Hoagland solution, the concentration of Ca^{2+} is 160 ppm which could be optimistic for the growth of the plant. It was thought that the addition of 100 μM Ca^{2+} in Hoagland solution led to the characteristic symptoms of calcium overdose. In the treatment of Cd^{2+} for two weeks, the plants were not dead even if they were showed severe inhibition of many physiological responses. However, in case of Ca^{2+}, the leaves were changed to almost bright yellow and led to die. Fortunately, Cd^{2+}-toxicity was greatly decreased by EGTA. This results were not expected as we thought that Ca^{2+} would compete into the transport to the plant with Cd^{2+} and therefore, Cd^{2+}-toxicity would be reduced. Therefore, it needs further study how calcium toxicities occur in growth and many physiological responses in term of the concentration of calcium and the exposure time of calcium.

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