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Effects of Aluminum on the Toxicity of Iron (II), Copper and Cadmium in Suspension-cultured Tobacco Cells

Yoko YAMAMOTO, Yi-Chieh CHANG, Kanji ONO
and Hideaki MATSUMOTO

The effects of aluminum (Al) on the cytotoxicity of ferrous iron (Fe (II)), copper (Cu) and cadmium (Cd) were studied. Log-phase cells were treated with either $FeSO_4$, $CuSO_4$ or $CdCl_2$ in the presence or absence of $AlCl_3$ (120 μM) for 18 h at pH 4.0. After the treatment, the viability was determined as relative growth of the metal-treated cells to the untreated control cells during the post-treatment culture. A single treatment with either Al, Fe (II) or Cd did not inhibit the growth at the metal concentrations up to 300 μM, 200 μM and 500 μM, respectively, whereas the growth was markedly inhibited at 15 μM Cu. Thus, the cells were relatively insensitive to Al, Fe (II) and Cd and sensitive to Cu. When cells were treated with both Fe (II) (120 μM) and Al (120 μM), the growth was significantly inhibited and the cellular contents of both Al and Fe increased synergistically. After the treatment with Cu (0 to 10 μM) with or without Al, the cells grew more vigorously when they were treated in the presence of Al, although the Cu contents of the cells were not altered by Al. The presence of Al during the treatment with Cd (0 to 2 mM) had no effect on the degree of growth inhibition by Cd. Thus, Al interacts with the toxicity of Fe (II), Cu and Cd in different manners; synergistic with Fe (II), antagonistic with Cu and apparently no effect on Cd.

Key words: Aluminum, Antagonistic, *Nicotiana tabacum*, Suspension culture, Synergistic

Research Institute for Bioresources, Okayama University, Kurashiki 710, Japan
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INTRODUCTION

Plant growth is limited on acid soils by various factors such as H^+ toxicity and metal toxicity (*eg.* Al, manganese, Fe). Al is a major constituent of mineral soils and Al ions interact with various kinds of inorganic and organic ligands and also directly attack roots. Although Al is recognized to be a major growth-limiting factor in acid soils, the molecular mechanism of Al toxicity has not been elucidated (for reviews, Foy 1988, Taylor 1988a, Rengel 1992).

The solubility of many metals is enhanced in acid soils. Thus, the interactions of metal ions, if any, may be important factors for their rhizotoxicity in acid soils. Only a few studies have been reported on the interactions between Al and other metals in whole plant systems (Liebig *et al.* 1942, Blevins and Massey 1959, Hiatt *et al.* 1963, Taylor 1988b).

In this study, we examined the effect of Al on the cytotoxicity of Fe (II), Cu and Cd in suspension-cultured tobacco cells. First, we found the synergistic effect of Al on Fe (II) toxicity (our unpublished data). Here, we substantiate this phenomenon under a slightly different experimental condition and extend our study to other metals, Cu and Cd.

MATERIALS AND METHODS

1. *Strain and media*

A cell line, SL, of *Nicotiana tabacum* L. cv Samsun was a gift from Dr. C. Nakamura, Kobe University, Japan (Nagai *et al.* 1989, Nakamura *et al.* 1988). Cells were grown in a modified Murashige-Skoog (MS) medium (pH 5.8) containing MS salts (Murashige and Skoog 1962), B5 vitamins (Gamborg *et at.* 1968), sucrose (30g / liter) and 2,4-D (1.5 μM) and subcultured at 7-day intervals by the transfer of 2 ml of the suspension of cells into 30 ml of fresh medium in a 100-ml Erlenmeyer flask. The cultures were shaken at 100 strokes per min at 25 ℃ in the dark. A modified MS medium deprived of phosphate (Pi), ethylenediaminetetra-acetic acid (EDTA), and $FeSO_4$ (medium A) at pH 4.0 was used for the treatment with metal. Medium A at pH 5.0 was used for washing cells before and after the treatment.

2. *Measurement of growth inhibition by metals*

Solutions containing 10mM of $AlCl_3$, $FeSO_4$ and $CuSO_4$ and 100 mM $CdCl_2$ were prepared using distilled water just before use and sterilized by passage through a filter (a pore size: 0.20 μm). For the treatment with metals, log-phase cells were washed three times with medium A (pH 5.0) and

were finally resuspended in medium A (pH 4.0) containing various concentrations of metals at a cell density of 100 mg fresh weight (FW) per 10ml and treated for 18 h on a rotary shaker at 100 rpm at 25 ℃. After the treatment, the cells (10-ml aliquots, corresponding to 100 mg FW at the start of treatment) were harvested by centrifugation, washed two times with medium A (pH 5.0), resuspended with 30 ml of the modified MS medium and cultured for 6 days. The cells (5-ml aliquots) were harvested by vacuum filtration on a filter paper and FW was measured. The relative growth (FW of metal-treated cells to FW of the untreated control cells) was determined.

3. *Determination of metal contents in the cells*

Cells were treated with or without metals as described above and 10-ml aliquots (corresponding to 100 mg FW at the start of the treatment) were harvested, and washed twice with medium A (pH 5.0). After addition of 0.5ml of the acid mixture (H_2SO_4 : HNO_3, 1 : 1, V / V), the cells were digested at 160 ℃ for 30 h. Metal concentrations were determined with an atomic absorption spectrophotometer (a graphite furnace atomizer, model Z-9000, Hitachi, Japan).

RESULTS

1. *Growth inhibition by Al in tobacco cells*

Cells were treated with $AlCl_3$ for 18h in the modified MS medium

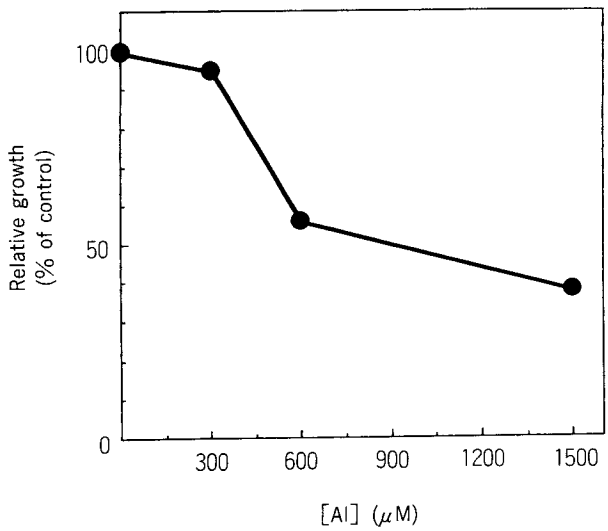

Fig. 1. Growth inhibition by Al. Cells were treated with various concentrations of $AlCl_3$ in medium A at pH 4.0 for 18 h. The relative growth of the treated cells to the untreated control cells was determined as described in Materials and Methods. Each value is the mean of two independent experiments.

deprived of Pi and EDTA-$FeSO_4$ (medium A), because Pi and EDTA reduced Al toxicity (our unpublished data), probably due to the formation of either insoluble aluminium phosphate or EDTA-Al chelate complex. Furthermore, the pH of the treatment medium was adjusted to 4.0 with KOH and HCl, because Al phytotoxicity was generally observed in solutions or soils with a pH below 5.0 (Foy, 1974). In our previous experiment, the medium pH was adjusted to 5.0 with 20 mM 2-(N-morpholino)-ethanesulfonic asid-Bis-tris propane, which caused nonspecific adsorption of Al to the cells and made it difficult to quantify the specific Al uptake (our unpublished data). At pH 4.0 which was used in this study, nonspecific adsorption of Al was apparently not observed (Yamamoto *et al.* data submitted). After the treatment, the viability of the cells was determined as the relative growth of the Al-treated cells to the untreated control cells during the post-treatment culture. As Figure 1 shows, the growth of the cells treated with $AlCl_3$ up to 300 μM was not inhibited and the growth of the cells treated with 600 or 1500 μM $AlCl_3$ was inhibited 50 to 60 %.

2. *Synergistic interaction between Al and Fe*

Treatment with up tp 200 μM $FeSO_4$ in medium A (pH 4.0) for 18 h did not inhibit the growth of the cells but that with 500 μM $FeSO_4$ inhibited it 30 %. Thus, the degree of toxicity of Fe (II) is similar to that of Al in the tobacco cells. Under this condition, the cells were treated with $FeSO_4$ (0 and 120 μM) in the presence or absence of $AlCl_3$ (120 μM) in medium A (pH 4.0) for 18 h, and the relative growth to the untreated control cells, Al content and Fe content in the cells were determined. Figure 2 (a) shows that the cells treated with either Al or Fe grew as vigorously as the control cells, whereas the growth inhibition was clearly observed when cells were treated with both Al and Fe. These findings indicate the synergistic action of Al and Fe to inhibit growth. Figure 2 (b) indicates that the Al content of the cells treated with Al [98 $\pm$ 4 (mean $\pm$ S.D.) nmole / 100 mg FW] was 2 times higher than that of the control cells (49 $\pm$ 4 nmole / 100 mg FW), whereas the Al content of the cells treated with both Al and Fe (1020 $\pm$ 69 nmole / 100 mg FW) was 21 times higher than that of the control cells. In the latter case, the increase in the Al content of the cells seemed to be due to absorption of Al into the cells instead of adsorption to the cell wall, because the Al content of the cells was not reduced after the digestion of the cell wall with Cellulase and Pectolyase (Yamamoto *et al.* data submitted). The Fe content was also significantly higher in the cells treated with Al and Fe (185 $\pm$ 12 nmole / 100 mg FW) than that either in the cells treated with only Fe (72 $\pm$ 5 nmole / 100 mg FW) or in the control cells (51 $\pm$ 11 nmole / 100 mg FW). These findings

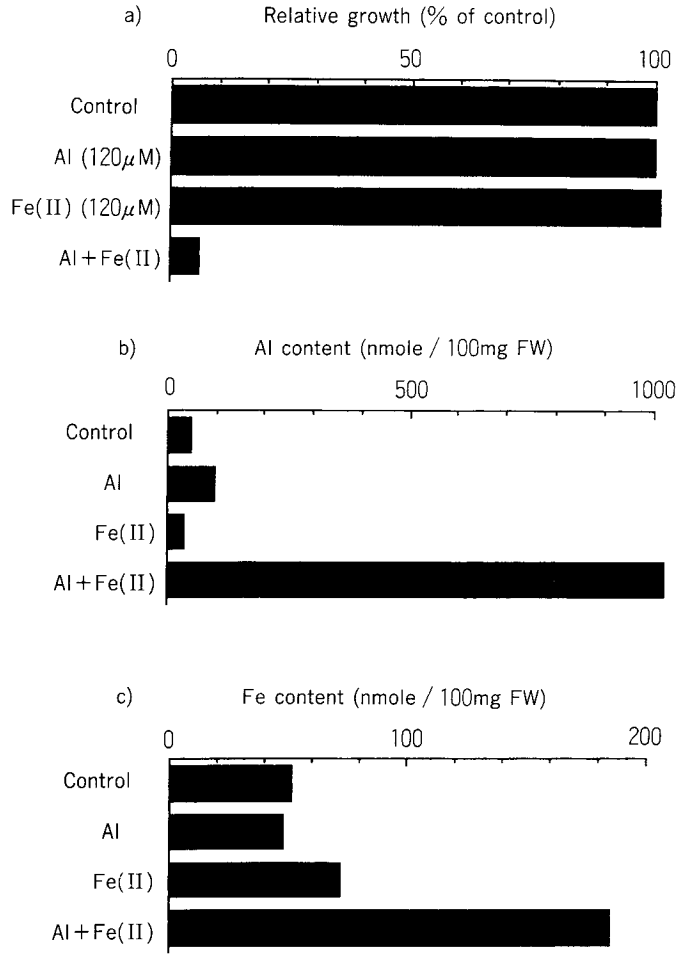

Fig. 2. Synergistic effects of Al and Fe (II) on cellular growth and on uptake of Al and Fe. Cells were treated with $FeSO_4$ (0 and 120 μM) in the presence or absence of $AlCl_3$ (120 μM) in medium A at pH 4.0 for 18 h. Relative growth and contents of Al and Fe in the cells were determined as described in Materials and Methods. Each value is the mean of triplicate cultures of one experiment.

suggest that the incorporation of Al and Fe into the cells occurs in a synergistic manner.

3. *Antagonistic interaction between Al and Cu*

The tobacco cells used in this paper were sensitive to Cu, and showed only 17 % growth as compared to that of the control cells after treatment with 7.5 μM $CuSO_4$ in medium A (pH 4.0) for 18 h (Fig. 3). Then, we examined the effect of Al on the Cu toxicity. The cells treated with $CuSO_4$ in the presence of $AlCl_3$ (120 μM) for 18 h grew better than the cells treated with $CuSO_4$ in the absence of $AlCl_3$ (Fig. 3). The growth of the cells treated

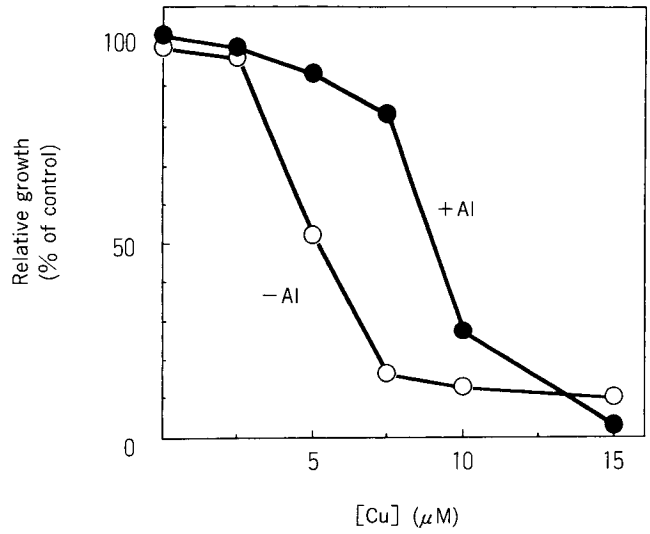

Fig. 3. Antagonistic effect of Al on Cu toxicity. Cells were treated with various concentrations of $CuSO_4$ (0 to 15 μM) in the presence (●) or absence (○) of $AlCl_3$ (120 μM) in medium A at pH 4.0 for 18 h. Relative growth was determined as described in Materials and Methods. Each value is the mean of two independent experiments.

with both 7.5 μM $CuSO_4$ and 120 μM $AlCl_3$ was 83 % of that of the control cells, which was 5 times higher than that (17 %) in the absence of Al. Thus, we conclude that the effect of Al on the Cu toxicity is antagonistic.

One possible explanation for this phenomenon may be the competition between Cu and Al for the adsorbing and / or absorbing sites in the cells as suggested by Blevins and Massey (1959) and Hiatt et al. (1963). However, this was not the case. Figure 4 shows that the Cu content of the cells treated

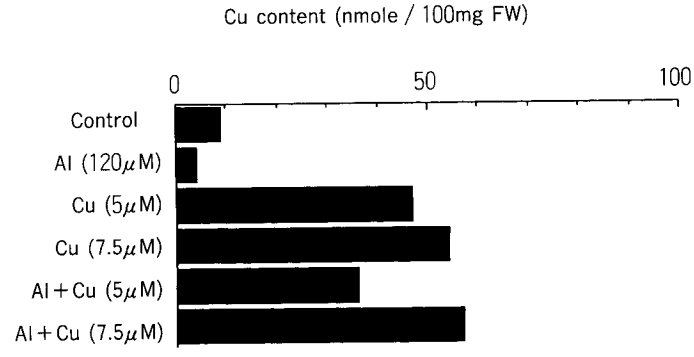

Fig. 4. Cu contents of the cells. Cells were treated with $CuSO_4$ (0, 5, 7.5 μM) in the presence or absence of $AlCl_3$ (120 μM) as described in Fig. 3. Cu content was determined as described in Materials and Methods. Each value is the mean of two independent experiments.

with Cu in the presence of Al was slightly lower at 5 μM $CuSO_4$ but almost the same level at 7.5 μM as that of the cells treated with Cu in the absence of Al. The Al content of the cells did not change either in the presence or absence of Cu (data not shown).

4. *Al is noneffective on Cd toxicity*

The tobacco cells were relatively insensitive to Cd, and the growth inhibition of the cells was not observed after the treatment even with 500 μM $CdCl_2$ in medium A (pH 4.0) for 18 h (Fig. 5). To examine the effect of Al on the Cd toxicity, the cells were treated with various concentrations of Cd with or without Al (120 μM) for 18 h. However, the degree of growth inhibition by Cd did not change by the presence of Al (Fig. 5), indicating that 120 μM $AlCl_3$ has no effect on the Cd cytotoxicity.

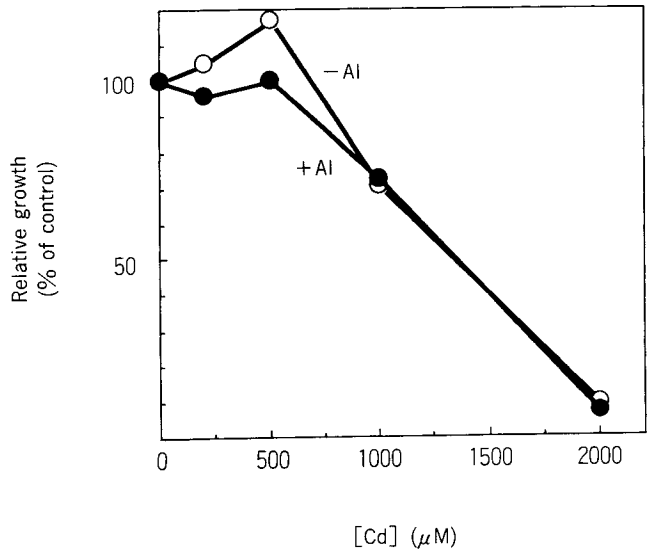

Fig. 5. Effect of Al on Cd toxicity. Cells were treated with various concentrations of $CdCl_2$ (0 to 2 mM) in the presence (●) or absence (○) of $AlCl_3$ (120 μM) in medium A at pH 4.0 for 18 h. Relative growth was determined as described in Materials and Methods.

DISCUSSION

The cytotoxicity of metal ions (Al, Fe (II), Cu and Cd) was examined in cultured tobacco cells of a nonchlorophyllic cell line, SL at an acidic pH. Judging from the lowest toxic concentration of each metal, the cells were sensitive to Cu but relatively insensitive to Al, Fe (II) and Cd (Figs. 1, 2, 3, 5). Under this condition, the effect of Al on the cytotoxicity of other metals

[Fe (II), Cu and Cd] was examined at the fixed Al concentration of 120 μM, at which Al had no effect on cellular growth (Fig. 1).

Al and Fe (II) inhibited growth synergistically. Under this condition, both metals, especially Al, were incorporated into the cells (Fig. 2). One possible explanation for this phenomenon is that it may be caused by the disruption of the membrane structure by Al and Fe (II). To support this, Gutteridge *et al.* (1985) reported that Al accelerated the peroxidation of ox-brain phospholipid liposomes induced by Fe (II) at an acidic pH. Since Al can not participate in the redox reactions due to a fixed oxidation number of III, they suggested that Al may act by causing a subtle rearrangement of membrane lipids that facilitates the peroxidative action of Fe (II). If the primary damage caused by Al is the alteration of structure and/or permeability of plasma membrane, Al may enhance incorporation of not only Fe but also other metal ions and may increase their cytotoxicity. However, in contrast to the synergistic effect on Fe (II) toxicity, the effects of Al on Cu toxicity and Cd toxicity are antagonistic and apparently no effect, respectively.

Cu toxicity was ameliorated in the presence of 120 μM $AlCl_3$ (Fig. 3). Liebig *et al.* (1942) also reported the ameliorative effect of Al on Cu toxicity in citrus. In tobacco cells, the Cu content of the cells was not altered significantly by Al (Fig. 4). This is in contrast to the results of Blevins and Massey (1959) and Hiatt et al. (1963) who showed that the uptake of Cu was clearly inhibited by the presence of low concentrations of Al and suggested that the antagonistic effect of Al on Cu adsorption may be caused by competition between these two ions for root adsorption sites.

The presence of Al had no effect on the Cd toxicity in tobacco cells (Fig. 5). On the contrary, an antagonistic interaction between Al and Cd on root elongation was reported in a Al-tolerant, Cd-sensitive race of *Holcus lanatus* (McGrath *et al.* 1980).

The present findings indicate that, although Al itself has phytotoxicity, synergistic or antagonistic interactions of Al with other metals should be also important factors of Al toxicity in acid soils. The mechanisms of the interactive effects of Al on Fe toxicity and Cu toxicity must be examined further to elucidate the molecular mechanism of Al cytotoxicity.

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タバコ懸濁培養細胞におけるアルミニウムの二価鉄，銅およびカドミウム毒性に対する影響

山本洋子・張　藝潔・小野寛治・松本英明

二価鉄 [Fe(II)]，銅(Cu)およびカドミウム(Cd)の細胞毒性に対するアルミニウム(Al)の効果について検討した．120μM $AlCl_3$の存在もしくは非存在下において，対数増殖期の細胞を$FeSO_4$，$CuSO_4$，$CdCl_2$で各々18時間，pH4.0で処理した．処理後の生存率は、処理後の細胞を増殖させたのちに，未処理細胞(コントロール)の増殖に対する金属で処理した細胞の相対増殖率で求めた．

Al，Fe(II)，Cdの単独処理による増殖阻害は，各々300μM，200μM，500μMの濃度まで観察されなかったが，Cuでは15μMで大きく阻害された．このように，タバコ細胞は相対的にAl，Fe(II)，Cdには感受性が低く，Cuには感受性が高かった．

細胞をFe(II)およびAlの両方で処理すると，増殖は著しく阻害され，AlおよびFeの細胞内含量も相乗的に増加した．Cuで処理した場合，Alを加えることにより逆に増殖率が増加した．しかし，細胞内Cu含量はAlの影響を受けなかった．Cd処理の場合，Alを加えてもCdによる増殖阻害の程度は変わらなかった．

このように，AlはFe(II)，Cu，Cd毒性に対して各々異なる相互作用を示し，Fe(II)に対し相乗効果を，Cuに対しては拮抗的な阻害を示したが，Cdに対してはみかけ上効果がなかった．

キーワード：アルミニウム，拮抗，*Nicotiana tabacum*，懸濁培養，相乗