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Excess copper induces an accumulation of putrescine in rice leaves

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Abstract. The effect of excess Cu^{2+} (sulfate salt) on putrescine (Put) accumulation in detached rice leaves was investigated. Cu^{2+} treatment increased Put concentrations in rice leaves under both light and dark conditions. This increase was more pronounced in the light than in the dark, suggesting the importance of illumination in Cu^{2+}-induced accumulation of Put in detached rice leaves. The photosynthetic electron transport inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea reduced Put accumulation induced by Cu^{2+} in the light. In darkness, Cu^{2+}-induced Put accumulation was more effective in the presence of glucose or sucrose than in their absence. In the light, Cu^{2+} also induced Put accumulation in six other rice cultivars. D-Arginine and α-methylornithine decreased concentrations of Put induced by Cu^{2+}, indicating that Cu^{2+} may affect the activities of both arginine decarboxylase and ornithine decarboxylase, enzymes responsible for the biosynthesis of Put.

Keywords: Copper; Putrescine; *Oryza sativa.*

Abbreviations: D-Arg, D-arginine; **DCMU**, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; **MO**, α-methylornithine; **Put**, putrescine; **Spd**, spermidine; **Spm**, spermine.

Introduction

Polyamines are low-molecular weight polycations present in all living organisms. Putrescine (Put) is the obligate precursor for spermidine (Spd) and spermine (Spm) in all systems studied so far (Evans and Malmberg, 1989). In response to various types of environmental stress, plants accumulate Put (Evans and Malmberg, 1989). In recent decades, industrial and urban activities have increased the deposition of heavy metals (such as copper) in the soil system (Tyler, 1972). Despite the apparent importance of polyamines, especially Put, in stress metabolism, little information is available on the effects of heavy metals on plant polyamine contents. Cd^{2+} treatment of detached oat and rice leaves resulted in a significant increase in Put concentrations (Hou and Kao, 1993; Weinstein et al., 1986). Agrawal et al. (1992) demonstrated that exposure of an unicellular green alga to mercury caused an increase in Put concentrations.

Copper is an essential element for plant growth (Arnon and Stout, 1939) and important in various biochemical process, but at toxic concentrations it interferes with numerous physiological processes (Fernandes and Henriques, 1991). Virtually no information has been reported on the effect of Cu^{2+} on the accumulation of polyamines. The objective of this study was to examine the effect of Cu^{2+} on polyamine concentrations in detached rice leaves.

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Materials and Methods

Rice (*Oryza sativa* L. cv. Taichung Native 1) was cultured as previously described (Kao, 1980). The apical 3-cm segments excised from the third leaves of 12-day-old seedlings were used. A group of 10 segments was floated in a Petri dish containing 10 mL deionized water or aqueous solution of tested compounds. Detached rice leaves were treated with 0.01~10 mM $CuSO_4$ at 27°C under light (40 µmol m^{-2} s^{-1}). For other experiments, incubation was carried out for various lengths of time in the light or in the dark.

For polyamine extraction, leaf segments were homogenized in 5% (v/v) perchloric acid. Polyamines were determined using HPLC after benzoylation as described previously (Chen and Kao, 1991). The amounts of polyamines were expressed as nmol g^{-1} fresh weight.

All experiments were performed three times and within each experiment treatments were replicated 4 times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Results

As indicated in Figure 1, the amount of Put increased significantly at concentrations of 1 and 10 mM Cu^{2+}. After 36 h incubation under light, treatment with Cu^{2+} at 10 mM, increased Put concentration 3- to 4-fold. No significant changes in Spd concentrations were observed in Cu^{2+}-treated detached rice leaves, whereas the concentrations of Spm were almost halved at the 1 and 10 mM Cu^{2+}.

Figure 2 shows the time course for Put concentrations in detached rice leaves treated with 10 mM Cu^{2+} in the light. Increases in Put concentrations, as a consequence of Cu^{2+} treatment, were detected 12 h after the start of incubation in the light. Increased duration of treatment with Cu^{2+} increased the concentrations of Put in detached rice leaves. In untreated leaves, Put concentration increased only slightly during the 48 h of incubation in the light. When detached rice leaves were exposed to Cu^{2+} in the dark (Figure 2), Put concentrations were found to increase up to 24 h, and decrease afterwards to their original levels at 48h. Untreated leaves, kept in the dark, showed a small continuous decrease in their Put concentrations. When dark-untreated detached rice leaves were re-illuminated, Put concentrations were increased (Figure 2). These results clearly demonstrated the importance of illumination

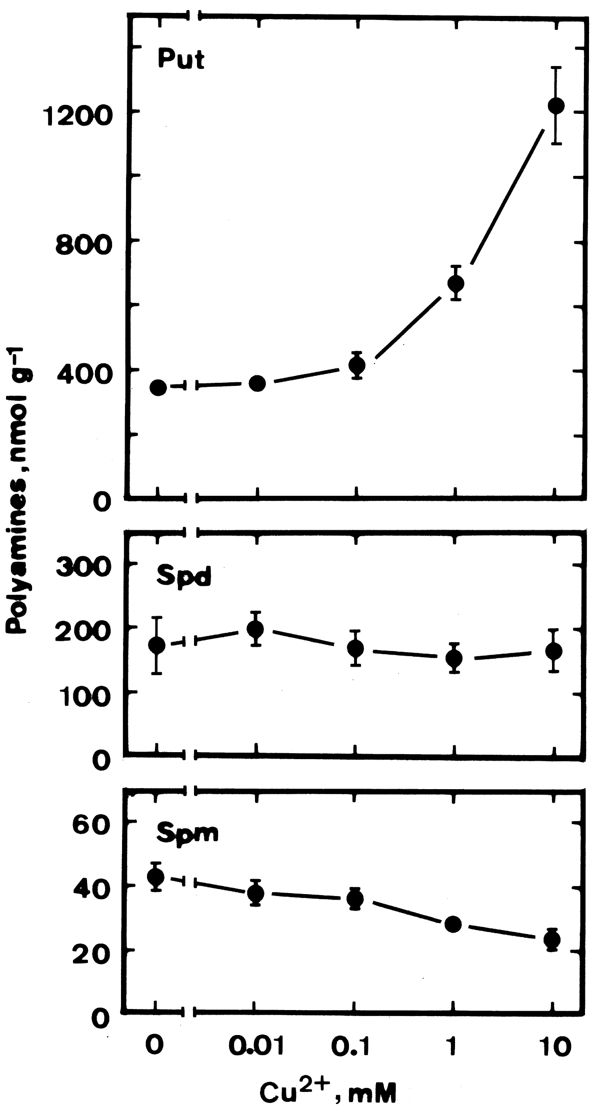

Figure 1. Effects of Cu^{2+} on the concentrations of polyamines in detached rice leaves. Detached rice leaves were floated on solutions of $CuSO_4$ at different concentrations for 36 h in the light. Bars show the standard errors and are contained within the symbols when not shown (n=4).

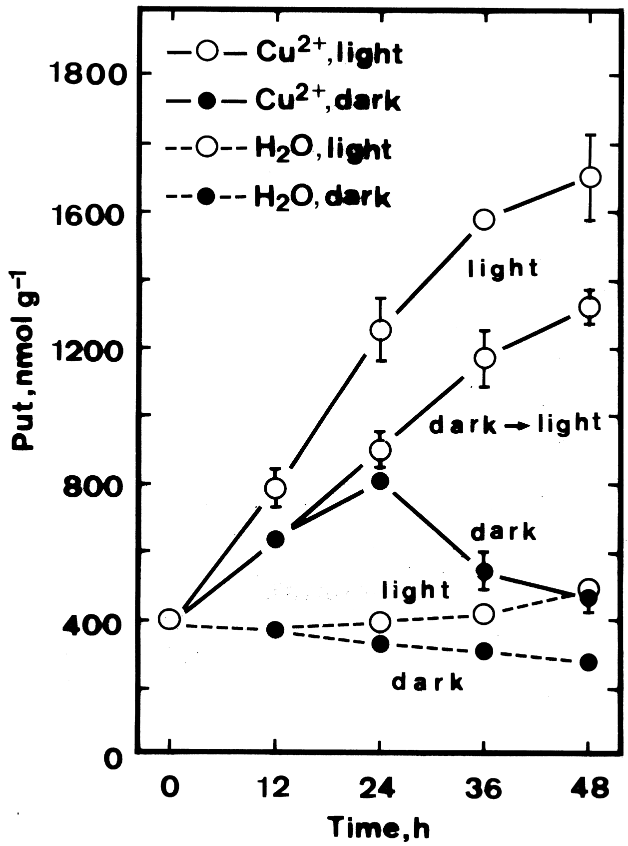

Figure 2. Time-course for Put accumulation in detached rice leaves treated with Cu^{2+} in the light or darkness. The concentration of $CuSO_4$ was 10 mM. Bars show the standard errors and are contained within the symbols when not shown (n=4).

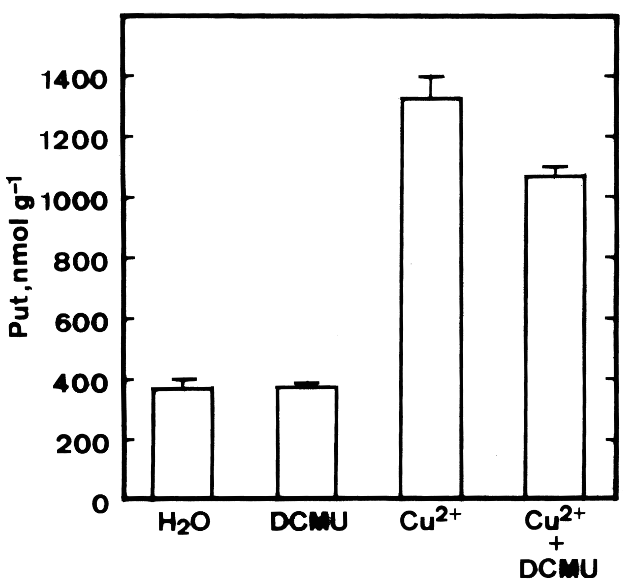

Figure 3. Effects of DCMU on Cu^{2+}-induced accumulation of Put in detached rice leaves in the light. Concentrations of $CuSO_4$ and DCMU were 10 and 0.1 mM, respectively. Put was determined 36 h after treatment. Bars indicate standard errors (n=4).

in Put accumulation induced by Cu^{2+}. The effect of light and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosynthetic electron transport, on the accumulation of Put by Cu^{2+} is shown in Figure 3. DCMU in the light reduced the accumulation of Put induced by Cu^{2+} but had no effect on water-treated leaves. Figure 4 showed that detached rice leaves incubated in the presence of glucose or sucrose under dark condition showed a greater Cu^{2+}-induced accumulation of Put than those incubated without sucrose or glucose.

Cu^{2+}-induced Put accumulation is not specific for the rice cultivar used in this study. Put concentrations were also increased by Cu^{2+} in the light in detached rice leaves of six other rice cultivars (Figure 5). It should be noted that Cu^{2+} is more effective in inducing Put accumulation in Japonica varieties (with an 8- to 13-fold increase) than in Indica ones (a 2- to 3-fold increase, Figure 5). It is clear that Put concentrations in Indica varieties are higher than in Japonica varieties in control leaves.

To investigate whether other divalent metals also increase the amount of Put in detached rice leaves, we tested Mg^{2+}, Zn^{2+}, Fe^{2+}, and Mn^{2+}. Results (Figure 6) indicate that, in the light, Fe^{2+} and Zn^{2+} also increased the Put concentrations in detached rice leaves. Fe^{2+} was found to be as effective as Cu^{2+} in inducing accumulation of Put in detached rice leaves. However, Mn^{2+} and Mg^{2+} had no effect on the amount of Put in detached rice leaves.

To characterize further the effect of Cu^{2+} on Put accumulation, inhibitors of its biosynthesis, such as D-arginine (D-Arg) and α-methylornithine (MO), were applied to Cu^{2+}-treated detached rice leaves, in the light. Results are shown in Figure 7. D-Arg, but not MO, significantly reduced the concentrations of Put in detached rice leaves in the absence of Cu^{2+}. However, both D-Arg and MO decreased the concentrations of Put induced by Cu^{2+}, indicating that Cu^{2+} may affect the activities of both arginine decarboxylase and ornithine decarboxylase, enzymes responsible for the biosynthesis of Put in higher plants (Evans and Malmberg, 1989). Since D-Arg has a more pronounced effect than MO in reducing Put accumulation induced by Cu^{2+}, arginine decarboxylase seems to be the major enzyme responsible for the accumulation of Put induced by Cu^{2+} in the light.

In this study, we used $CuSO_4$ as the source of Cu^{2+}. We also found that $CuCl_2$ induced a 3- or 4-fold increase in Put concentration in detached rice leaves in the light (data not shown), indicating that the results reported here are indeed attributable to Cu^{2+}.

Discussion

Accumulation of polyamines has been shown to be a response to accelerated growth in plants (Altman and Bachrach, 1981; Flores, 1990), and it can also occur under a variety of stress conditions. Increase in Put concentrations in plants was observed under conditions of high salinity, low pH, ammonium addition, potassium and magnesium deficiency, osmotic stress, chilling, cadmium

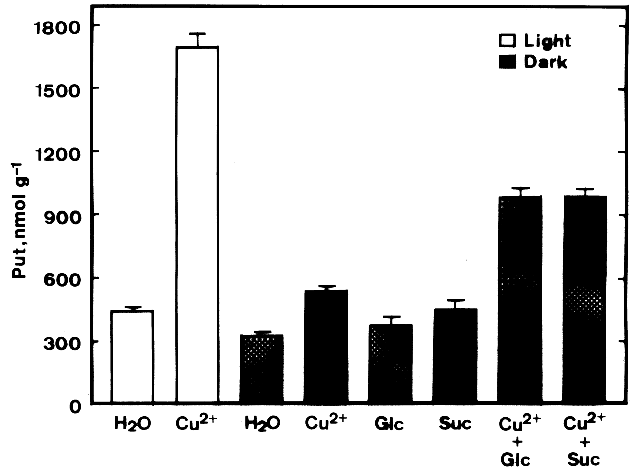

Figure 4. Effects of glucose and sucrose on Cu^{2+}-induced accumulation of Put in detached rice leaves in darkness. The concentration of $CuSO_4$, glucose and sucrose were 10, 50 and 50 mM, respectively. Put was determined 36 h after treatment. Bars indicate standard errors (n=4).

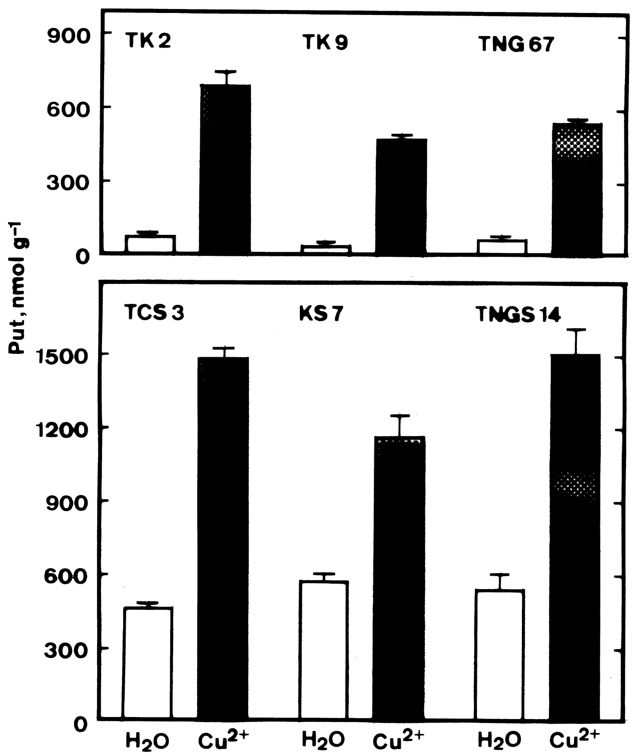

Figure 5. Cu^{2+}-induced Put accumulation in detached rice leaves from 6 rice cultivars. Those in the upper panel are Japonica varieties whereas those in the lower panel are Indica varieties. TK2, Tai Ken 2; TK9, Tai Ken 9; TNG67, Tainung 67; TCS3, Taichung Sen 3; KS7, Kaoshiung Sen 7; TNGS14, Tainung Sen 14. Detached rice leaves were treated with either water (open column) or 10 mM $CuSO_4$ (shaded column) in the light. Put was determined 36 h after treatment. Bars indicate standard errors (n=4).

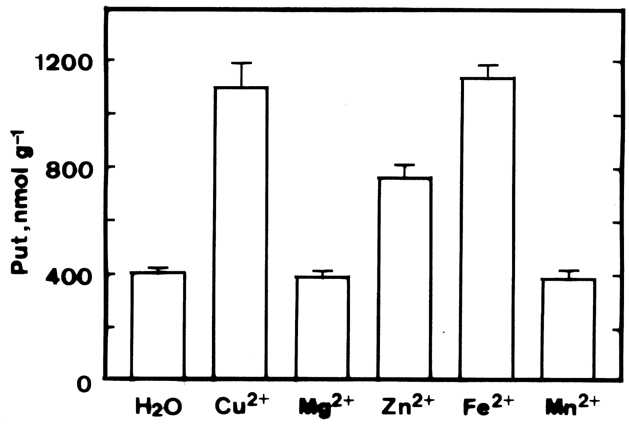

Figure 6. Effects of divalent metals on concentrations of Put in detached rice leaves. Leaves were floated with sulfate salts of various metals (10 mM) in the light for 36 h. Bars indicate standard errors (n=4).

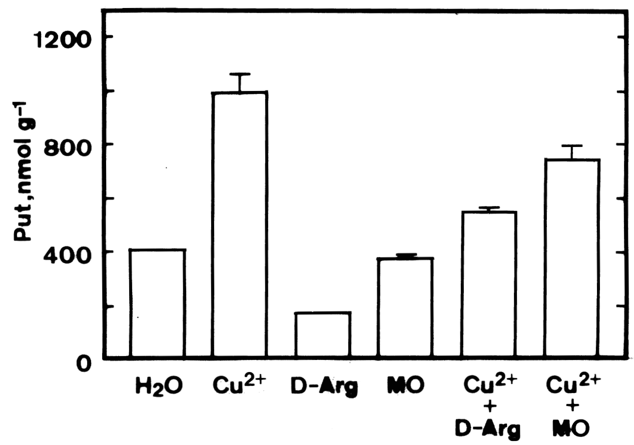

Figure 7. Effects of D-Arg and MO in the absence or presence of Cu^{2+} on the amount of Put in detached rice leaves. Detached rice leaves were incubated in the light for 36 h in D-Arg (5 mM) or MO (5 mM) in the absence or presence of $CuSO_4$ (10 mM). Bars indicate standard errors (n=4).

exposure, and ozone treatment (Evans and Malmberg, 1989).

We report here that Put accumulated in rice leaves following exposure to excess Cu^{2+}. This is the first report, to our knowledge, of a Cu^{2+}-induced change in polyamine concentrations and suggests that Cu^{2+}-induced Put accumulation is a stress response similar to that observed under a variety of other stress conditions.

The requirement for de novo Put biosynthesis in Cu^{2+}-treated rice leaves was demonstrated by its concentration decrease when D-Arg or MO were added to rice leaf segments. These results indicate that Cu^{2+} interferes with the activities of both arginine decarboxylase and ornithine decarboxylase. Weinstein et al. (1986) investigated the effects of Cd^{2+} on Put, Spd and Spm concentrations in oat and bean leaves. They found that Cd^{2+} treatment up to 16 h in the light or dark resulted in a large increase in Put concentrations and that arginine decarboxylase was the enzyme responsible for Put increase.

Of particular interest is the finding that Cu^{2+}-induced accumulation of Put in detached rice leaves is strongly light dependent. It seems that Put accumulation induced by Cu^{2+} in detached rice leaves in the light is modulated by photosynthetic activity. Since DCMU only partially reduced Cu^{2+}-induced Put accumulation in the light, and neither glucose nor sucrose increased the Put concentrations in Cu^{2+}-treated detached rice leaves in darkness to the same extent as Cu^{2+} in the light, one may conclude that light is required for one or more processes other than photosynthesis for maximum Put accumulation.

While it is clear that a series of defined stresses results in large increase in Put concentrations, we cannot at present conclude if this is part of a protective response or the syndrome of toxicity observed under such conditions. The more recent evidence suggests the latter is more likely (Flores, 1990).

The toxic nature of high intracellular Put concentrations has been demonstrated in salt stressed horse bean and pea plants (Strogonov et al., 1972) and in potassium deficient barley (Coleman and Richards, 1956). Recently, we also reported that growth inhibition in suspension-cultured rice cells under potassium and phosphate deprivation was closely associated with Put accumulation (Shih and Kao, 1996; Sung et al., 1994). Since an increase in endogenous Put concentrations does not enhance degradation of chlorophyll and protein (Chang and Kao, 1997), generally considered a symptom of Cu^{2+} toxicity, and since addition of D-Arg and MO to Cu^{2+}-treated leaf segments, which caused a reduction of Put accumulation (Figure 7), did not reduce the toxicity caused by Cu^{2+} (data not shown), it seems that Put accumulation is not involved in the sequence of events leading to Cu^{2+} toxicity. This conclusion is supported further by the observation that Cu^{2+} toxicity occurs at a concentration of 0.01 mM (data not shown), too low to induce Put accumulation (Figure 1). Thus, the functional role of Put accumulation induced by Cu^{2+} merits further investigation.

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過量銅離子處理會誘導水稻切離葉片 putrescine 之累積

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本研究係探討過量銅離子（硫酸鹽型式）處理對水稻切離葉片 putrescine (Put) 含量之影響。不論是光線或黑暗下，銅離子處理會導致 Put 含量之增加。然而，光線下 Put 含量之增加大於黑暗下，顯示光線對 Put 含量增加之重要性。以光合作用電子傳遞之抑制劑 [3-(3,4-dichlorophenyl)-1,1-dimethylurea] 處理可有效的降低光線下銅離子處理所誘導之 Put 累積。在黑暗下添加葡萄糖或蔗糖可增加銅離子所誘導之 Put 累積。在光線下，銅離子亦可誘導其他六種水稻切離葉片 Put 之累積。D-Arginine 與 α-methylornithine 降低銅離子所誘導之 Put 累積，顯示銅離子可增加合成 Put 之酵素，亦即 arginine decarboxylase 與 ornithine decarboxylase 之活性。

關鍵詞：銅；Putrescine；水稻。