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Polyamines in relation to ammonium-inhibited growth in suspension-cultured rice cells

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Abstract. The role of endogenous polyamines in affecting the growth inhibition of rice cells fed with ammonium was investigated. When ammonium was used as the sole nitrogen source, the growth of rice cells, as judged by dry or fresh mass, was markedly inhibited. Ammonium-fed rice cells had a higher level of putrescine (Put) than the control cells (supplied with both nitrate and ammonium). However, ammonium-fed cells had much lower levels of spermidine (Spd) and spermine (Spm) than the control. The growth inhibition of rice cells induced by ammonium could not be recovered by the addition of Spd and Spm. D-Arginine or α-methylornithine, inhibitors of Put biosynthesis, caused a reduction in the level of Put in ammonium-fed rice cells, but was unable to reverse the growth inhibition of rice cells fed with ammonium. It was concluded that endogenous polyamines might not play a role in influencing the ammonium-inhibited growth of rice cells.

Keywords: Ammonium; Cell growth; *Oryza sativa*; Putrescine; Spermidine; Spermine.

Introduction

Polyamines occur ubiquitously in animals, plants, and prokaryotes, and their roles in affecting growth, development, and stress metabolism have been reviewed (Evans and Malmberg, 1989). In both animal cells and bacteria, changes in polyamine levels have been shown to accompany growth (Herbst and Snell, 1948; Tabor et al., 1982; Cohen et al., 1984). Polyamine levels and plant growth rates are positively correlated under a wide variety of conditions in which high levels of polyamines are associated with rapidly growing tissues (Evans and Malmberg, 1989). In contrast, Ozawa and Tusjii (1993) demonstrated that soybean plants accumulated sufficient putrescine (Put) and spermidine (Spd) in their nodules to inhibit the growth of bacteroids of *Bradyrhizobium japonicum* strain 138NR. In a recent work, Rea et al. (1995) also observed that Spd inhibited growth of maize roots.

Plant cell cultures do not usually grow on ammonium as a sole source of nitrogen (Gamborg and Shyluk, 1970; Behrend and Mateles, 1976; Fukunaga et al., 1978; Kaul and Hoffman, 1993). It has been shown that plants accumulate Put when grown with ammonium instead of nitrate (Hohlt et al., 1970; Klein, 1979; Smith, 1984). Recently, we demonstrated that Put accumulation was a factor causing growth inhibition of rice cells under potassium deficiency (Sung et al., 1994). However, it is not known whether ammonium-inhibited growth of rice cells is also mediated via Put accumulation.

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The present investigation was designed to examine the role of endogenous polyamines in affecting the growth inhibition of rice cells fed with ammonium. We present data showing that endogenous polaymines play no role in the control of ammonium-inhibited growth of rice cells.

Materials and Methods

Rice (*Oryza sativa* cv. Tainan 5) suspension cultures were initiated from calli derived from immature embryo (Yu et al., 1991) and subsequently maintained at 25°C by weekly transfers to fresh liquid medium of Murashige and Skoog (1962) supplemented with 3% sucrose and 5 μM 2,4-dichlorophenoxyacetic acid. Polyamines and inhibitors of Put biosynthesis were sterilized by filtration before adding to the culture medium.

Suspension-cultured cells were collected by filtration through a 400-mesh nylon sieve, and blot-dried on paper towels. The growth of rice cells was measured by fresh mass and dry mass. Dry mass determination was made after drying at 80°C for 48 h. For polyamine determination, the collected cells (about 50 mg fresh mass) were homogenized in 5 ml of 5% perchloric acid. Polyamine levels were determined using high performance liquid chromatography after benzoylation as described previously (Chen and Kao, 1991).

For all measurements, each treatment in an experiment was performed four times, and all experiments were performed three times. Similar results and identical trends were obtained each time; therefore, only one set of data from a single experiment is presented.

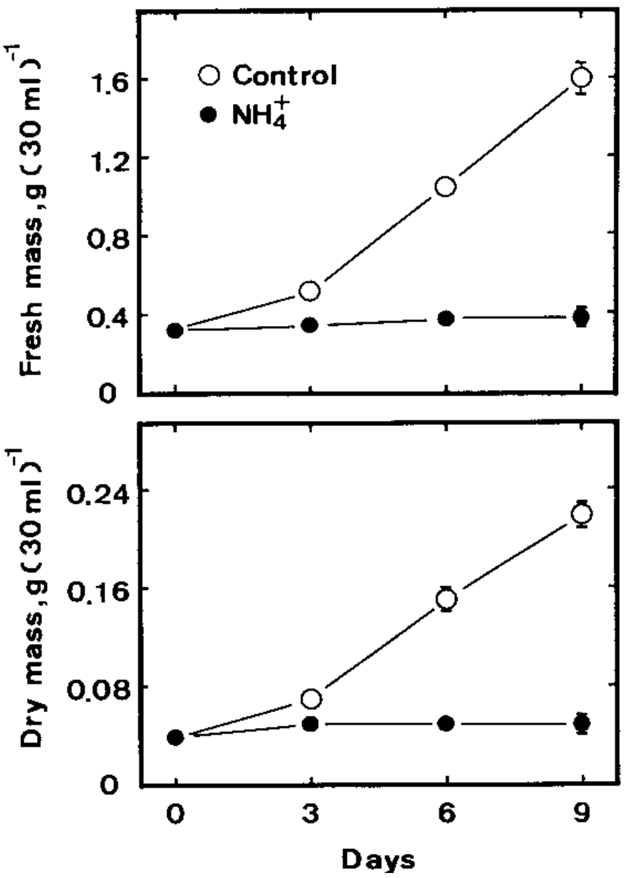

Figure 1. Changes in the growth of rice cells cultured with Murashige and Skoog medium containing 40 mM nitrate and 20 mM ammonium (control) and 60 mM ammonium only. Vertical bars represent standard errors (n=4). Only those standard errors larger than symbol size are shown.

Results and Discussion

Figure 1 shows the changes in dry or fresh mass of rice cells cultured with Murashige and Skoog medium containing both nitrate and ammonium or ammonium only. When ammonium was used as the sole nitrogen source, the growth of rice cells, was almost completely inhibited. However, the growth in the control cells (supplied with both nitrate and ammonium) increased linearly with increasing duration of culture.

To characterize the role of polyamines in ammonium-inhibited growth of rice cells, the levels of polyamines in the control cells and ammonium-fed cells were determined. The level of Put in the control cells increased at Day 3 and decreased thereafter (Figure 2). Ammonium-fed rice cells had higher level of Put than the control cells as reported in other plant species (Hohlt et al., 1970; Klein et al., 1979; Smith, 1984). The levels of Spd and Spm in the control cells increased during the first 3 and 6 days of culture, respectively, but decreased at Day 9 (Figure 2). On the other hand, ammonium-fed rice cells had a much lower level of Spd and Spm than the control cells (Figure 2).

If low levels of Spd and Spm were responsible for the growth inhibition in rice cells fed with ammonium, then the growth of these cells should recover by the addition of Spd and Spm. However, no such recovery was observed (data not shown).

Inhibitors of polyamine biosynthesis have been widely used to change the levels of endogenous polyamines in plant tissues. In order to elucidate the role of Put accumulation in ammonium-inhibited growth of rice cells, we tested the effects of inhibitors of Put biosynthesis, D-arginine (D-Arg) and α-methylornithine (MO), on the level of Put and the growth of rice cells fed with ammonium (Figures 3 and 4). Both D-Arg and MO decreased the lev-

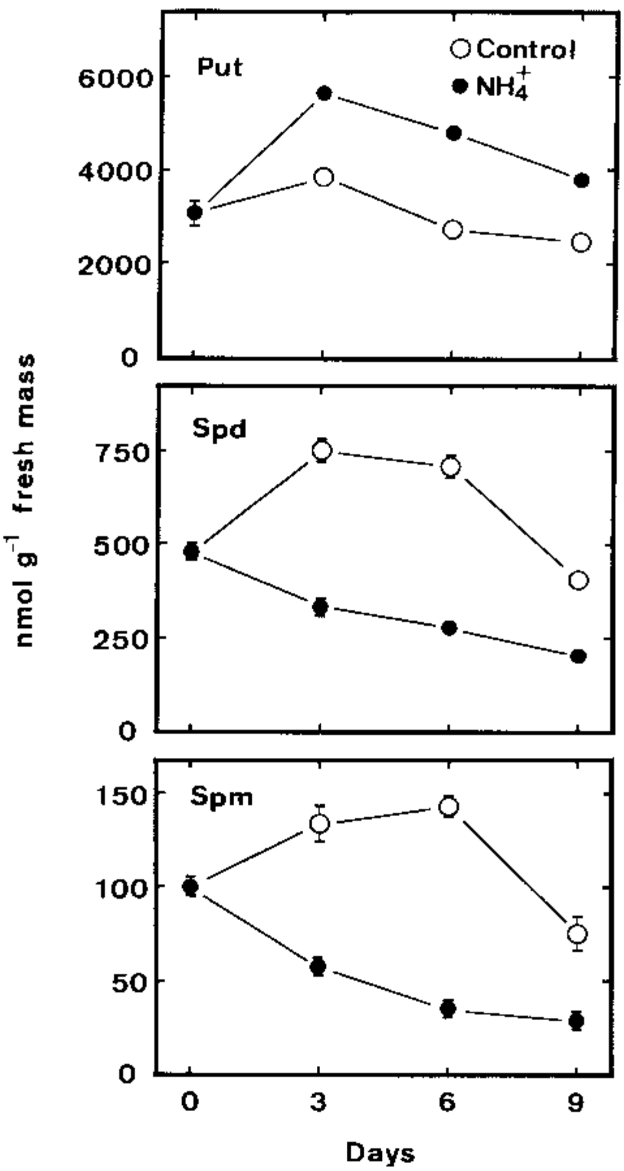

Figure 2. Changes in levels of polyamines in rice cells cultured with Murashige and Skoog medium containing 40 mM nitrate and 20 mM ammonium (control) and 60 mM ammonium only. Vertical bars represent standard errors (n=4). Only those standard errors larger than symbol size are shown.

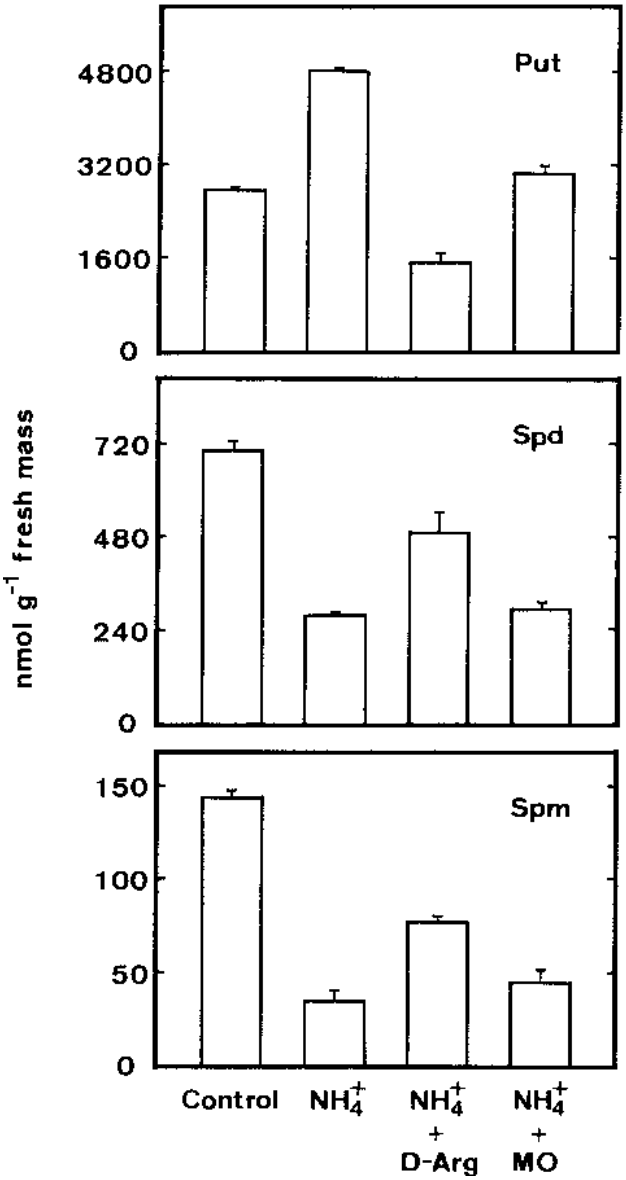

Figure 3. Effect of D-arginine (D-Arg) and α-methylornithine (MO) on the levels of polyamines in rice cells cultured with Murashige and Skoog medium containing 60 mM ammonium only. The concentration of D-Arg and MO was 5 mM. Polyamines in rice cells were determined after 6 days of treatment. Vertical bars represent standard errors (n=4).

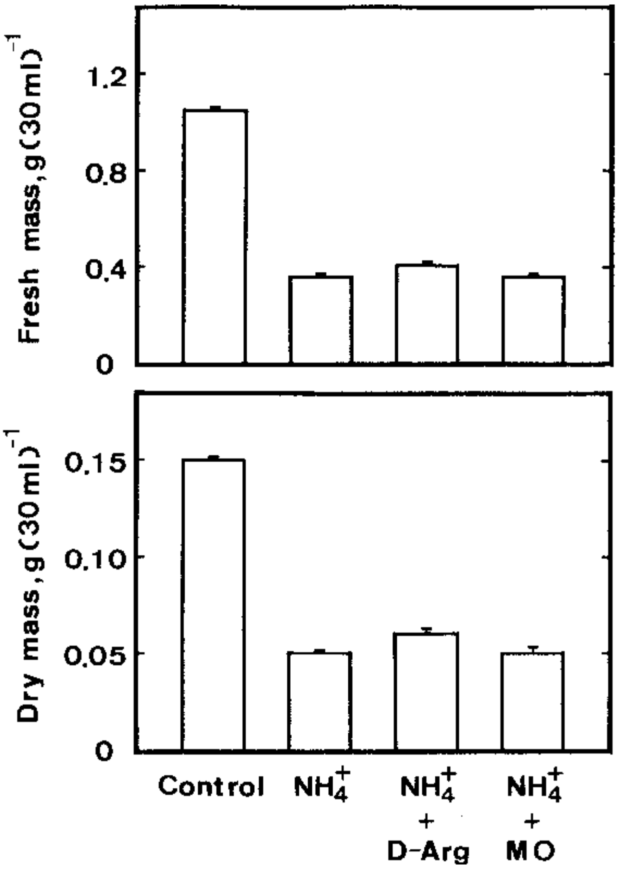

Figure 4. Effect of D-arginine (D-Arg) and α-methylornithine (MO) on growth inhibition of rice cells by ammonium (60 mM). The concentration of D-Arg and MO was 5 mM. The growth of rice cells was measured after 6 days of treatment. Vertical bars represent standard errors (n=4).

els of Put in ammonium-fed rice cells (Figure 3). However, niether D-Arg nor MO treatment resulted in a reverse of growth inhibition in ammonium-fed rice cells (Figure 4). These results suggest that Put accumulation is not a factor causing growth inhibition of ammonium-fed rice cells. It should be noted that both D-Arg and MO had no effect on the growth of the control cells, although they significantly reduced the level of Put (data not shown).

Previously, we demonstrated that Put accumulation is a factor responsible for growth inhibition of rice cells under potassium deficiency (Sung et al., 1994). Why then is Put accumulation not a factor in this case? The difference might be attributable to the level of Put accumulated in the cells. Put level in the potassium-deprived rice cells was about 4 times greater than that in the control (Sung et al., 1994). However, Put level in ammonium-fed rice cells was only 1.5 times greater than the control (Figure 2). Presumably, the ammonium-fed rice cells did not accumulate sufficient Put to inhibit the growth of rice cells, and the inhibition might be the result of other factors such as ammonium toxicity.

MO treatment had little effect on the levels of Spd and Spm in ammonium-fed rice cells (Figure 3). However, D-Arg treatment resulted in an increase in the levels of Spd and Spm in ammonium-fed rice cells, but did not reverse the growth inhibition of rice cells fed with ammonium (Figures 3 and 4). These results further support our suggestion that lower levels of Spd and Spm are not responsible for the ammonium-inhibited growth of rice cells.

In conclusion, our results indicate that the changes in polyamine levels are unlikely to be the factor responsible for ammonium-inhibited growth of rice cells.

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