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Growth Inhibition in Suspension-Cultured Rice Cells under Phosphate Deprivation Is Mediated through Putrescine Accumulation[1]

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The effects of phosphate deprivation on the growth and polyamine levels of suspension-cultured rice (*Oryza sativa*) cells were investigated. When rice suspension cells were deprived of phosphate, cell growth was markedly inhibited. Phosphate deprivation resulted in a higher putrescine level and lower spermidine and spermine levels in rice suspension cells. The growth of rice cells cultured in the absence of phosphate did not recover as a result of spermidine and spermine addition. D-Arginine and α-methylornithine, inhibitors of putrescine biosynthesis, caused a reduced level of putrescine in rice suspension cells cultured under phosphate deprivation. The growth of rice cells cultured in the absence of phosphate was completely recovered after the addition of D-arginine but not α-methylornithine. Our results indicate that putrescine accumulation is a factor causing growth inhibition of suspension-cultured rice cells under phosphate deprivation.

Polyamines are low-molecular-weight polycations and are present in a all living organisms. In both animal cells and bacteria, changes in polyamine concentration have been shown to accompany growth (Herbst and Snell, 1948; Tabor et al., 1982; Cohen et al., 1984). Polyamine levels and plant growth rates have been positively correlated in a wide variety of conditions in which high levels of polyamines are associated with rapidly growing tissues (Evans and Malmberg, 1989).

In response to various types of environmental stress, plants accumulate polyamines, especially PUT. PUT accumulation in plants has been reported in response to deficiencies of inorganic ions, especially potassium and magnesium ions (Smith, 1984). One of the most important yet least available mineral nutrients for plant growth is P. Under phosphate deficiency, PUT was found to accumulate in tobacco and barley plants (Takahashi and Yoshida, 1960; Sinclair, 1967).

Suspension-cultured cells offer a convenient system with which to study the correlation of polyamines with cell growth without other developmental implications. The present investigation was designed to study the time courses of polyamine levels during phosphate deprivation of rice (*Oryza sativa*) cells and their correlation with cell growth.

MATERIALS AND METHODS

Rice (*Oryza sativa* cv Tainan 5) suspension cultures were initiated from calli derived from immature embryos (Yu et al., 1991). The rice grain was sterilized with 1% sodium hypochlorite and 1 drop of Tween 20 for 20 to 30 min and then washed extensively with distilled water. The embryo was excised and then placed scutellum side up on Murashige-Skoog agar medium (Murashige and Skoog, 1962) containing 0.8% agar, 3% Suc, and 5 μM 2,4-D. The pH was adjusted to 5.8 before autoclaving. The embryo culture was incubated at 25°C under white light (42 μmol m^{-2} s^{-1}). Four weeks later, initiated calli were transferred to liquid Murashige-Skoog medium containing 3% Suc and 5 μM 2,4-D. Approximately 500 mg of fresh mass of callus were cultured in 30 mL of medium in a 125-mL Erlenmyer flask. The suspension culture was shaken on a reciprocal shaker at 120 rpm and incubated at 25°C under a constant irradiance of 42 μmol m^{-2} s^{-1}. Established suspension-cultured cells were subcultured every 7 d by transferring about 0.5 mL of cells into 30 mL of fresh liquid Murashige-Skoog medium in a 125-mL flask. The cell cultures were maintained growing logarithmically and 7-d-old cultures were used for initiation of all of the indicated experiments. To cause phosphate deficiency, rice cells were cultured in the absence of a P source. Polyamines and inhibitors of polyamine biosynthesis were filter sterilized before being added 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 48 h at 80°C. Pi was extracted with perchloric acid (2.5%) and determined by the spectrophotometric method of Yoshida et al. (1972). The protein level was determined according to the method of Bradford (1976). 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 HPLC after benzoylation as described previously (Chen and Kao, 1991).

For all measurements, each treatment was repeated four times. All experiments described here were re-

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Abbreviations: MO, α-methylornithine; PUT, putrescine; SPD, spermidine; SPM, spermine.

peated three times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

RESULTS AND DISCUSSION

Figure 1 shows the changes in Pi level in suspension-cultured rice cells in the presence or absence of exogenous phosphate. The Pi level in the control cells increased at d 2 and subsequently declined. The decrease in Pi level in the control cells at the later stage is possibly due to the dilution effect caused by the rapid growth of cells (Fig. 2). The Pi level in phosphate-deprived cells was always lower than that in the control cells (supplied with sufficient phosphate) during 8 d in culture. Phosphate deprivation markedly inhibited cell growth, as judged by fresh or dry mass (Fig. 2). The growth of rice cells with a sufficient supply of phosphate increased with increasing duration of culture (Fig. 2). However, only slight growth was observed in rice cells under phosphate deficiency. The protein level in phosphate-deprived cells was also lower than that in the control cells (Fig. 2).

To characterize the role of polyamines in the growth of rice cells under phosphate deficiency, the levels of polyamines in the cells cultured in the presence and absence of phosphate were determined. The chromatographic analysis indicated the presence of PUT, SPD, and SPM in suspension-cultured rice cells. Cadaverine and diaminopropane were not observed in rice cells. The level of PUT in the control cells remained unchanged for the first 6 d of culture but increased at d 8 (Fig. 3). Phosphate-deprivated rice cells had a much higher level of PUT than the control cells. In contrast, phosphate deprivation resulted in lower levels of SPD and SPM than the control cells (Fig. 3).

If the lower levels of SPD and SPM are responsible for the growth inhibition in rice cells under phosphate deprivation, then phosphate-deprived rice cells might be expected to recover their growth rate by the addition of SPD and SPM. However, no such recovery was observed (Fig. 4).

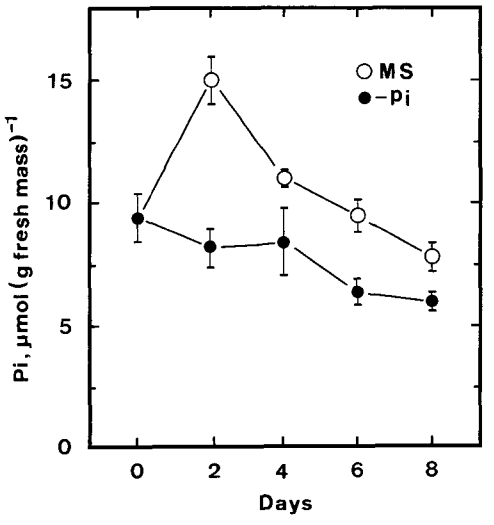

Figure 1. Changes in Pi level in rice cells cultured with sufficient phosphate (MS) and under phosphate deprivation (−Pi). Vertical bars represent SES (n = 4).

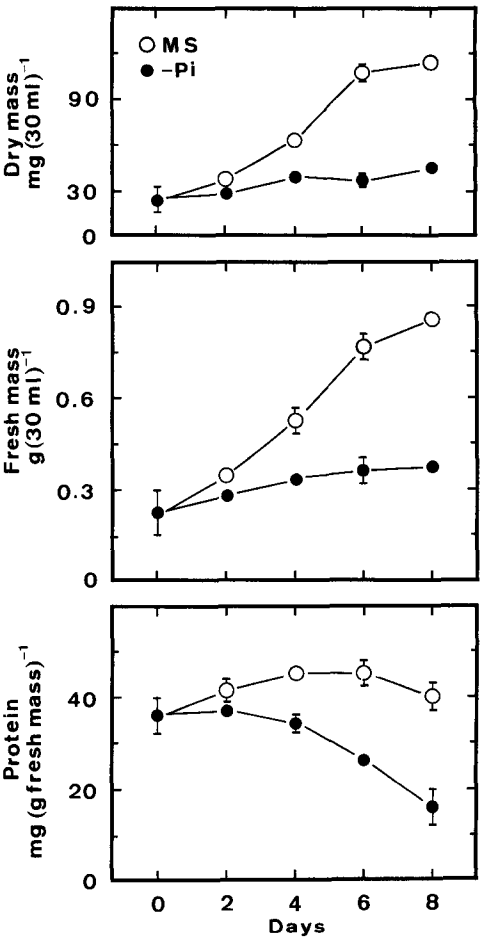

Figure 2. Changes in the growth and protein level of rice cells cultured with sufficient phosphate (MS) and under phosphate deprivation (−Pi). Vertical bars represent SES (n = 4). Only those SES larger than the symbols are shown.

Since PUT accumulated in rice cells under phosphate deficiency, PUT may be a factor causing growth inhibition in rice cells under phosphate deprivation. If this suggestion is correct, PUT is expected to inhibit the growth in control rice cells. Addition of 10 mM PUT to control rice cells resulted in about 20% reduction in cell growth (data not shown). Our results are in contrast to the general notion that in many systems including plants, microorganisms, and animals polyamine treatment has resulted in growth promotion (Evans and Malmberg, 1989).

Inhibitors of polyamine biosynthesis are commonly used to change the level of intracellular polyamines in plant tissues. To elucidate the role of PUT accumulation in growth inhibition of rice cells under phosphate deprivation, we tested the effect of inhibitors of PUT biosynthesis (D-Arg and MO) on the intracellular level of PUT and the growth of rice cells under phosphate deprivation (Figs. 5 and 6). Both D-Arg and MO decreased the levels of PUT induced by phosphate deprivation (Fig. 5). Although the

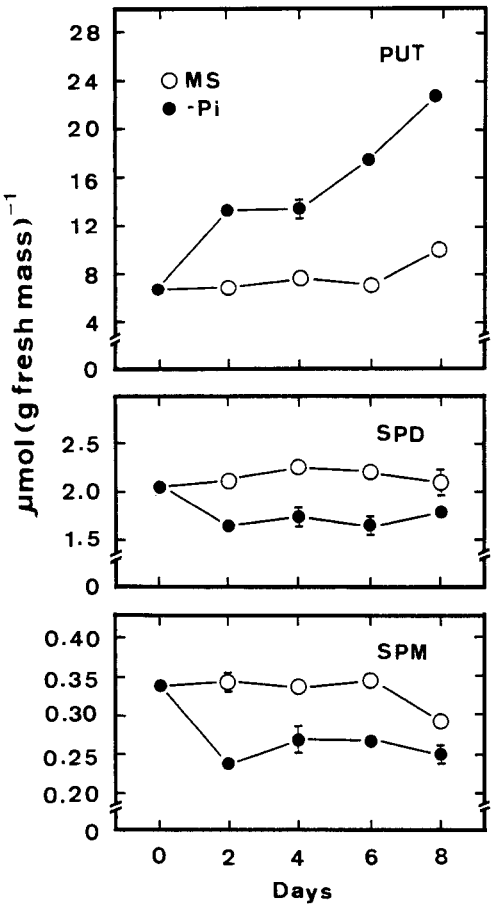

Figure 3. Changes in levels of polyamines in rice cells cultured with sufficient phosphate (MS) and under phosphate deprivation (−Pi). Vertical bars represent SES (n = 4). Only those SES larger than the symbols are shown.

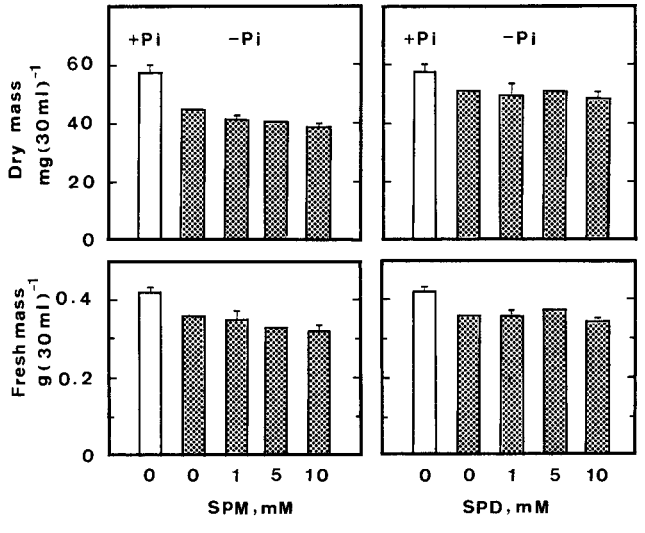

Figure 4. Effects of SPD and SPM on the growth of rice cells under phosphate deprivation. The growth of rice cells was measured after 4 d of treatment. Vertical bars represent SES (n = 4).

activities of Arg decarboxylase and Orn decarboxylase, enzymes responsible for the biosynthesis of PUT (Evans and Malmberg, 1989), were not measured, the results suggest, although indirectly, that phosphate deprivation may affect the biosynthesis of PUT.

D-Arg treatment resulted in a complete recovery of growth in rice cells under phosphate deprivation, but MO had no effect on growth recovery (Fig. 6). D-Arg and MO treatments, which decreased the level of PUT in the control cells, had no effect on the growth of the control rice cells (data not shown). Although MO significantly reduced the accumulation of PUT induced by phosphate deprivation, MO-treated phosphate-deprived rice cells still had a higher level of PUT than the control cells (Fig. 5). This would explain why MO is unable to restore the growth inhibited under phosphate deprivation. Figure 5 also shows that MO treatment resulted in an increase in SPD and SPM levels. These results further support the conclusion that the lower levels of SPD and SPM under phosphate deprivation are not responsible for growth

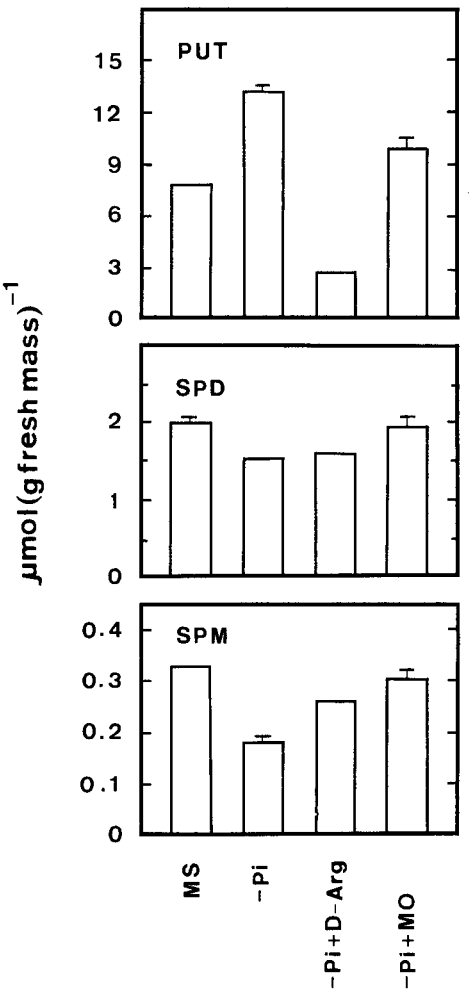

Figure 5. Effect of D-Arg and MO on the levels of polyamines in rice cells under phosphate deprivation. The concentration of D-Arg and MO was 5 mM. Polyamines in rice cells were determined after 4 d of treatment. Vertical bars represent SES (n = 4).

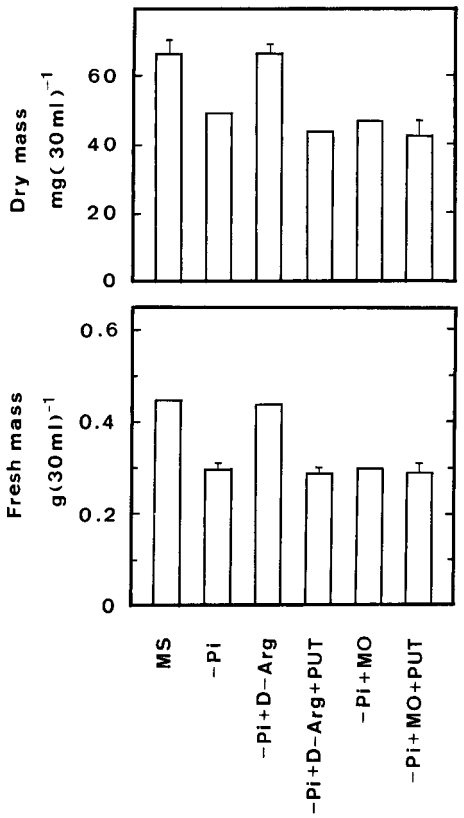

Figure 6. Effect of D-Arg and MO on the growth of rice cells under phosphate deprivation. The concentration of D-Arg and MO was 5 mM. The growth of rice cells was measured after 4 d of treatment. Vertical bars represent SEs (n = 4).

inhibition of rice cells. These results suggest that PUT accumulation is a factor related to growth inhibition of rice cells under phosphate deprivation. This conclusion is further supported by the observation that growth recovery in phosphate-deprived rice cells by D-Arg was reversed by the addition of PUT (Fig. 6).

In view of the findings reported here, it would appear that PUT is unlikely to be a growth factor of rice cells. On the contrary, PUT in excess of the level normally found in rice cells could be a factor inhibiting growth of rice cells.

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