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Sucrose-starvation-induced changes in polyamine and abscisic acid levels of suspension-cultured rice cells

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Abstract. We investigated the influence of sucrose starvation on the growth and on the polyamine and abscisic acid (ABA) levels of suspension-cultured rice cells. When the cells were deprived of sucrose, cell growth was strongly inhibited. Sucrose starvation resulted in lower polyamine levels and higher ABA levels in rice suspension cells than in the control cells. Our results indicate that the decrease in polyamine levels and the increase in ABA levels are not associated with growth inhibition in sucrose-starved suspension-cultured rice cells.

Keywords: Abscisic acid; Cell growth; *Oryza sativa*; Polyamines; Sucrose starvation.

Abbreviations: ABA, abscisic acid; **Put**, putrescine; **SCV**, settled cell volume; **Spd**, spermidine; **Spm**, spermine.

Introduction

Polyamines are low-molecular weight polycations, and are present in all living organisms. In response to various types of environmental stress, plants accumulate polyamines, especially putrescine (Put). Putrescine accumulation has been reported in response to K^+ and Mg^{2+} deficiencies (Basso and Smith, 1974; Sung et al., 1994), exposure to heavy metal salts (Hou and Kao, 1993; Weinstein et al., 1986), osmotic stress (Chen and Kao, 1993b; Flores and Galston, 1984), acid stress (Chen and Kao, 1993a; Young and Galston, 1983), and SO_2 pollution (Priebe et al., 1978). Abscisic acid (ABA), a plant hormone with multiple physiological influences on growth and differentiation (Zeevaart and Creelman, 1988), has also been shown to accumulate in response to water stress (Zeevaart and Creelman, 1988), salt and osmotic stress (Creelman and Zeevaart, 1985; Mizrahi et al., 1970; 1972; Ribaut and Pilet, 1991), flooding (Zhang and Davies, 1987), high temperature (Daie and Campbell, 1981), and chilling (Zeevaart and Creelman, 1988).

Several lines of evidence indicate that polyamines are involved in the regulation of plant growth (Altman, 1989). It is usually accepted that ABA is a potent growth inhibitor, although in a few cases it may promote growth (Milborrow, 1974).

Carbohydrate starvation is a fact of life for most plants. Carbohydrate starvation can occur in plants because of the shedding of leaves, a reduction in the amount of photosynthetic tissue or of the water supply, extreme temperatures, or a transfer to heterogrowth conditions (Tassi et al., 1992). A deeper knowledge of metabolic changes caused by carbon starvation is necessary for a better understanding of the mechanism of carbon starvation tolerance—we thus chose to use sucrose starvation to monitor the levels of ABA and polyamines. Suspension-cultured cells offer a convenient system in which to study the correlation of polyamines and ABA with cell growth without other developmental implications. The present investigation was designed to study the time courses of polyamine and ABA levels during sucrose starvation of rice cells and their correlation with cell growth.

Materials and Methods

Rice (*Oryza sativa* L. cv. Tainan 5) suspension cultures were initiated from calli derived from immature embryo (Yu et al., 1991) and were maintained by weekly transfers to fresh, liquid Murashige and Skoog (1962) medium supplemented with 3% (w/v, 88 mM) sucrose and 5 μM 2,4-D. The cultures were maintained at 25°C. To cause sucrose starvation, rice cells were cultured in the absence of sucrose. Polyamines and ABA 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 collected cells were quick-frozen in liquid nitrogen and stored at -70°C until use.

The growth of rice cells was measured by settled cell volume (SCV). For polyamine determination, the collected cells 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). Enzyme-linked immunosorbent assay (ELISA) was used to measure ABA levels. The procedures for extraction and separation of

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ABA have been described previously (Chen and Kao, 1993b). Polyamine and ABA levels are expressed as μmol and pmol per g fresh mass, respectively.

For all measurements, each treatment was repeated four times. All experiments described here were repeated three times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Results and Discussion

Cell growth in the present investigation comprises the increase in cell size and cell number. Sucrose starvation markedly inhibited the growth of suspension-cultured rice cells (Figure 1). The growth of rice cells with a sufficient supply of sucrose increased linearly with increasing duration of culture, but almost no growth was observed in rice cells under sucrose starvation.

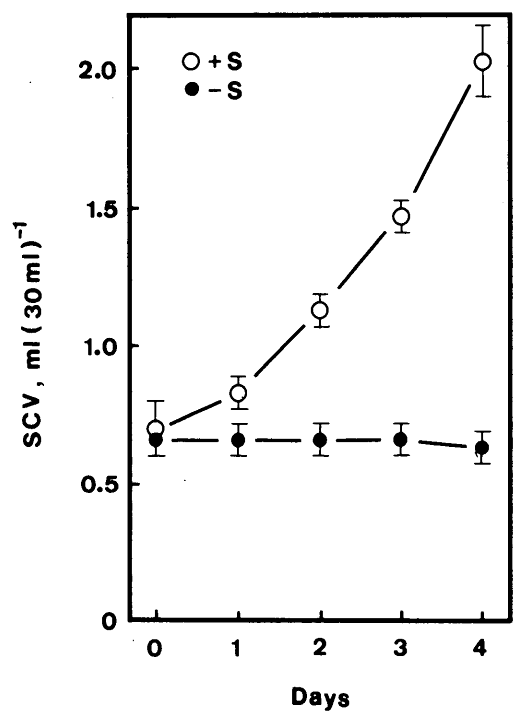

Figure 1. Changes in the growth of rice cells cultured with sufficient sucrose (+S) and under sucrose starvation (-S). Bars are standard errors (n=4).

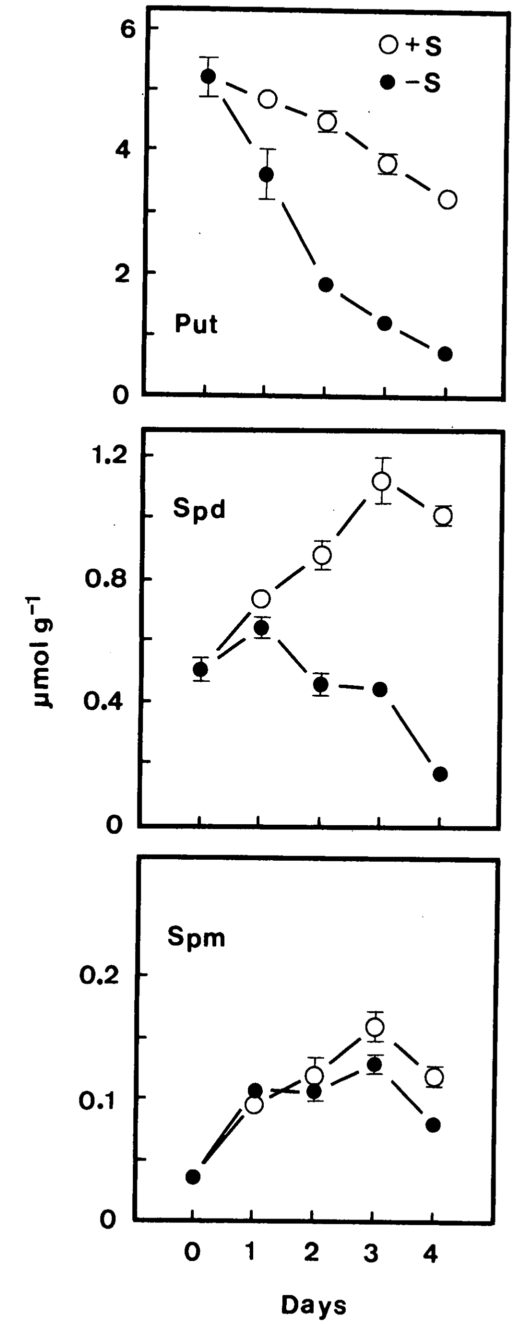

Figure 2. Changes in levels of polyamines in rice cells cultured with sufficient sucrose (+S) and under sucrose starvation (-S). Bars are standard errors (n=4).

To characterize the role of polyamines in the growth of rice cells under sucrose starvation, we determined the levels of polyamines in cells cultured in the presence and absence of sucrose. Major polyamines in rice suspension cells were Put, spermidine (Spd), and spermine (Spm). Neither cadaverine nor diaminopropane was observed in rice cells. Spermidine and Spm levels in the control cells (supplemented with sucrose) increased with culture duration (Figure 2). In contrast, the Put level in control cells decreased with increasing duration (Figure 2). During the course of culture, the levels of Put and Spd in cells cultured in the absence of sucrose were always lower than those in the control cells (Figure 2). For the first 2 days, Spm levels increased in the presence and absence of sucrose, but sucrose starvation resulted in a lower level of Spm at days 3 and 4.

Polyamines have been reported to be required for the growth of suspension-cultured rice cells (Manoharan and Gnanam, 1992). If lowered levels of polyamines are responsible for the growth inhibition in sucrose-starved rice cells, then such cells can be expected to recover their growth when polyamines are added. No such recovery, however, was observed (Figure 3). Our results suggest that the decrease in polyamine levels is not associated with growth inhibition in sucrose-starved rice cells, but is part of the overall expression of sucrose starvation in rice cells.

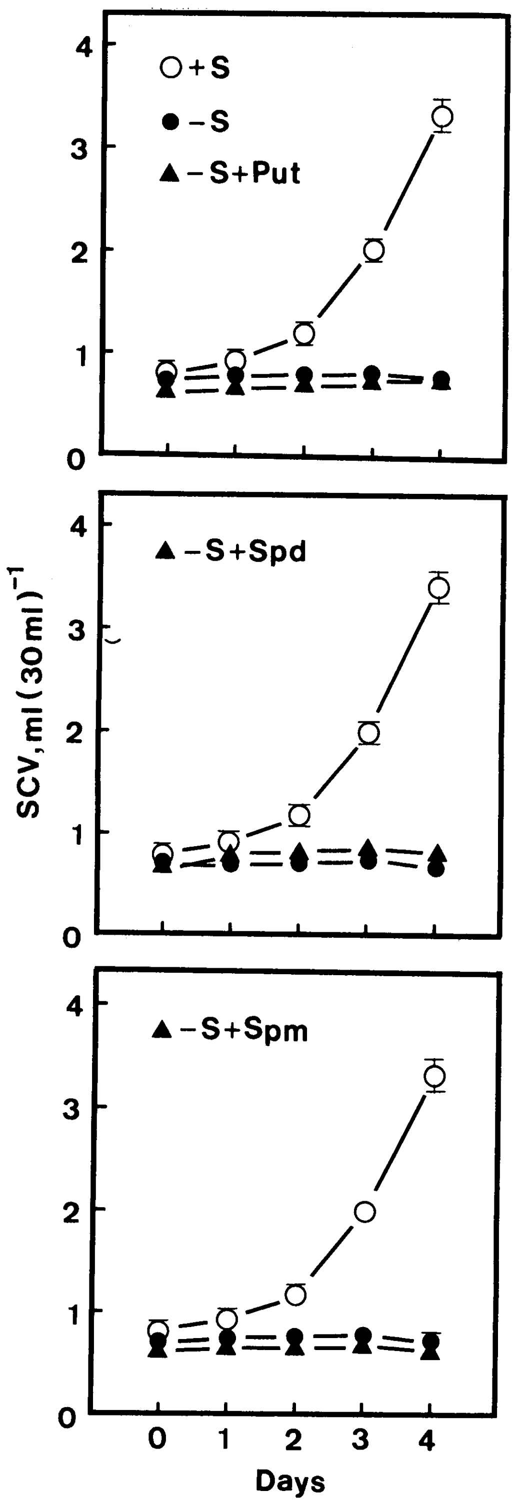

Figure 3. Influence of polyamines on the growth of rice cells inhibited by sucrose starvation (-S). The concentration of polyamines was 5 mM. Bars are standard errors (n=4).

It is usually accepted that ABA is a potent growth inhibitor (Milborrow, 1974). To characterize the role of ABA in the growth of sucrose-starved rice cells, we determined the levels of ABA in cells cultured in the presence and absence of sucrose. For the first 2 days, ABA levels increased both in the presence and absence of sucrose, but

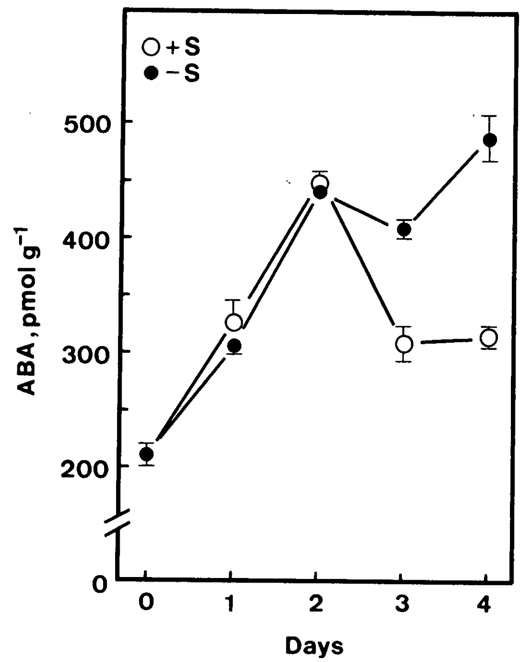

Figure 4. Changes in levels of ABA in rice cells cultured under sufficient sucrose (+S) and sucrose starvation (-S). Bars are standard errors (n=4).

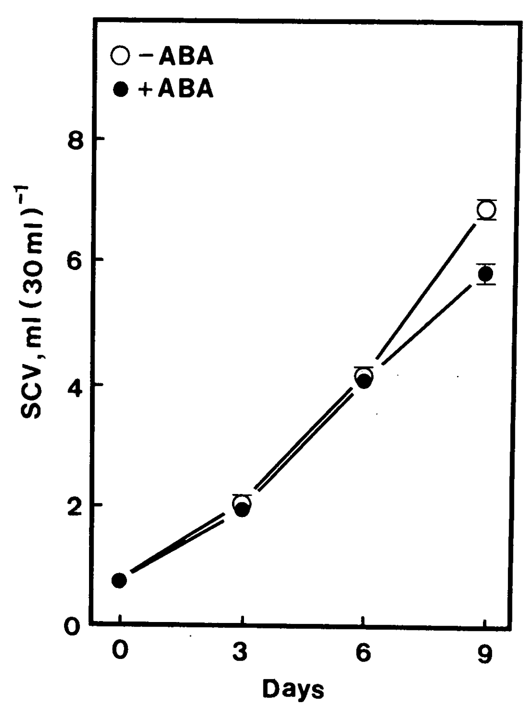

Figure 5. Influence of ABA on the growth of rice cells cultured with sufficient sucrose. The concentration of ABA was 10 μM. Bars are standard errors (n=4).

sucrose starvation resulted in a higher level of ABA at days 3 and 4 (Figure 4). Since high levels of ABA induced by sucrose starvation were only observed during the later period of culture, ABA is unlikely to be the factor responsible for growth inhibition of sucrose-starved rice cells. This conclusion is further supported by the observation that the addition of ABA (10 μM) to control rice cells caused only slight reduction in cell growth at day 9 (Figure 5).

The important findings of the present investigation are that sucrose starvation increased the levels of ABA in rice cells. To our knowledge, this is the first evidence that sucrose starvation results in a high level of ABA in cells. It seems that sucrose starvation can act like certain other stresses (chilling, flooding, salinity, water and osmotic stress, and high temperature) and cause a high level of ABA. For any cell to grow, there must be some source of carbon. When the cells have no energy source in the medium, they have nothing to metabolize for energy or for generating a carbon skeleton to build up cell materials. Consequently, the starved cells enter into senescence. This explains the observed data on the changes in levels of ABA and polyamines.

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蔗糖缺乏對水稻懸浮培養細胞多元胺與離層酸含量之影響

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本研究主要探討水稻懸浮培養細胞在缺少蔗糖狀況下，細胞內多元胺與離層酸含量之變化；同時並探討細胞內多元胺與離層酸含量變化與細胞生長間之關係。水稻細胞培養於缺少蔗糖的狀況下，細胞生長受抑制，多元胺含量降低，同時離層酸含量增加。添加多元胺無法恢復蔗糖缺少所抑制的細胞生長，同時，外加離層酸只能少許抑制對照細胞之生長。我們的結果認爲，多元胺含量的降低與離層酸含量的增加與蔗糖缺少所抑制的細胞生長無關。

關鍵詞：離層酸；細胞生長；水稻；多元胺；蔗糖缺乏。