Plant Growth Regulation **13**: 133–136, 1993.

Changes in putrescine accumulation induced by agents affecting cytosolic pH in detached rice leaves

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Received 10 September 1992; accepted 4 November 1992

Key words: cytosolic pH, *Oryza sativa*, putrescine, rice

Abstract

Effects of compounds that influenced cytosolic pH on the level of putrescine in detached rice leaves were examined. Permeant weak acids, isobutyric acid and propionic acid, increased the level of putrescine in detached rice leaves. Procaine and trisodium citrate, known to be permeant weak bases, on the other hand, decreased the level of putrescine. It seems possible that the level of putrescine in detached rice leaves is regulated by the cytosolic pH.

1. Introduction

The accumulation of putrescine in plants has been demonstrated in potassium deficiency, with ammonium excess and with acid feeding [4,8,11,12,13,14]. The results with acid stress and ammonium nutrition are consistent with the hypothesis that putrescine accumulation compensates for lowered pH in the cytoplasm of the stressed cells.

Reliable measurement of cytosolic pH in plant cells is still difficult, especially in mature cells in which most of the cellular volume is occupied by the vacuole. Modification of cytosolic pH has been achieved, however, by using weak acids. Weak acids such as isobutyric acid permeate into cells in their undissociated form and dissociate subsequently, releasing protons and acidifying the cytosol [1,7,9]. On the other hand, weak bases such as procaine raise the cytosolic pH [5,9]. If change of cytosolic pH does occur prior to the accumulation of putrescine, then agents that influenced cytosolic pH are expected to affect the level of putrescine. The present investigation describes the effects of agents affecting cytosolic pH on the accumulation of putrescine in detached rice leaves.

2. Materials and methods

2.1 Plant material

Seedlings of rice (*Oryza sativa* cv. Taichung Native 1) were grown in hydroponic culture as described previously [3]. The apical 3 cm of the third leaves of 12-day-old seedlings were used for the experiments.

2.2 Incubation conditions

Ten segments of rice leaves were floated on 10 ml of test solution in a Petri dish. All samples were kept at 27 °C in the dark. Permeant weak acids, isobutyric acid and propionic acid, and permeant weak bases, procaine and trisodium citrate, were dissolved in distilled water, with pH about 3.5, 3.2, 8.6 and 7.5, respectively.

2.3 Determination of free putrescine

At the indicated times (see results), the leaf samples were used to determine the level of free putrescine. For putrescine determination, leaf samples were homogenized in 5 ml of 5% perchloric acid. Putrescine level was quantitated using high performance liquid chromatography after benzoylation

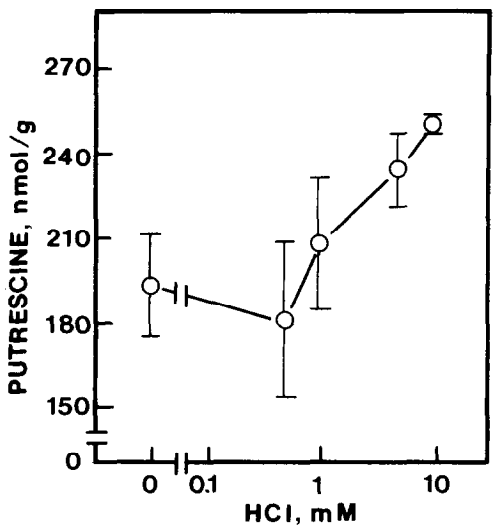

Fig. 1. Relationship between concentration of HCl and the level of putrescine in detached rice leaves. Putrescine was determined after 8 h of incubation in darkness. Bars indicate standard errors (n = 3).

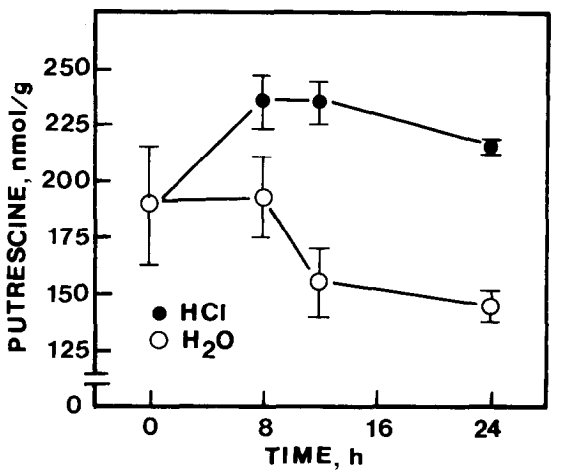

Fig. 2. Changes with time in the level of putrescine in detached rice leaves treated with HCl. Detached rice leaves treated with either water or 5 mM HCl in darkness. Putrescine was determined at the times indicated. Bars indicate standard errors (n = 3).

as described previously [2]. The level of putrescine was expressed as nmol g^{-1} fresh weight.

3. Results and discussion

In order to understand whether the increase in the level of putrescine also occurs in detached rice leaves when exposed to an external supply of excess hydrogen ions, detached rice leaves were treated with 0.5–10 mM HCl. As indicated in Figure 1, the level of putrescine increases significantly at concentrations of 5 and 10 mM HCl. The effect of this acid was further investigated by exposing control and HCl-treated rice leaf segments to dark period of various lengths. Increase in the level of putrescine was detected 8 h after application (Fig. 2). It is clear that detached rice leaves, similar to other plant systems [11,12], have the capability to maintain high level of putrescine on acid feeding.

Suresh et al. [12] reported that HCl (10 mM) treatment nearly doubled putrescine levels in *Cucumis sativus* cotyledons. However, we observed only about 30% increase of putrescine level by acid feeding. This discrepancy is most likely due to different plant materials used.

The effects of the concentration of isobutyric acid and procaine on the levels of putrescine in detached rice leaves in darkness are presented in Figure 3. Increasing the concentration of isobutyric acid from 1 mM to 5 mM progressively enhances the accumulation of putrescine by detached rice leaves. No significant increase was observed when isobutyric acid concentration was increased to 10 mM. Contrary to the effect of isobutyric acid, procaine, which is known to raise cytosolic pH, decreases the level of putrescine when applied at a concentration of 2.5 mM. No further decrease of the level of putrescine could be detected when this concentration was increased to 5 mM or 10 mM.

Figure 4 shows the changes with time in the level of putrescine in detached rice leaves treated with 5 mM isobutyric acid or 5 mM procaine. An increase and a decrease of the level of putrescine were observed at 8 h after the start of incubation in isobutyric acid and procaine, respectively. Propionic acid, also known to acidify cytoplasm, and trisodium citrate, known as an alkalizing agent [6], also increased and decreased the level of putrescine, respectively (data not shown).

The important finding in the present investigation was that the level of putrescine in detached rice leaves can be modified by agents affecting cytosolic pH. Such a regulation of the level of putrescine provides, though indirectly, evidence that putrescine accumulation in acid stress and with ammonium nutrition is most likely triggered by lowering pH in the cytoplasm in the stressed cells. However, this

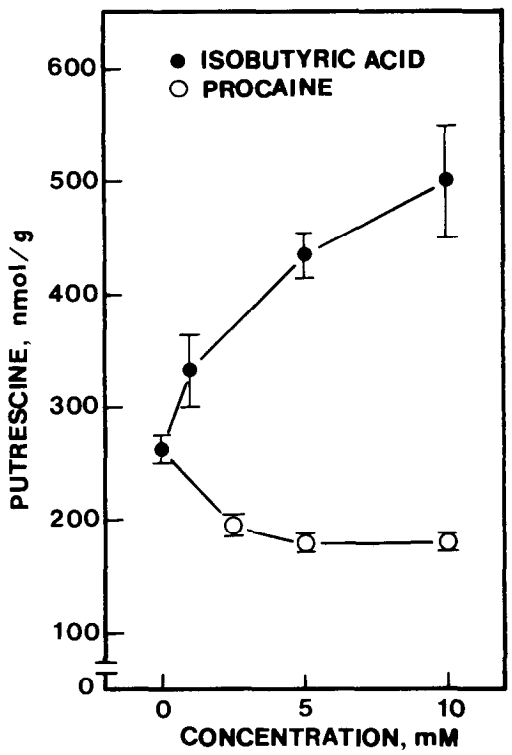

Fig. 3. Relationship between concentration of isobutyric acid or procaine and the level of putrescine in detached rice leaves. Putrescine was determined after 8 h of incubation in darkness. Bars indicate standard errors (n = 3).

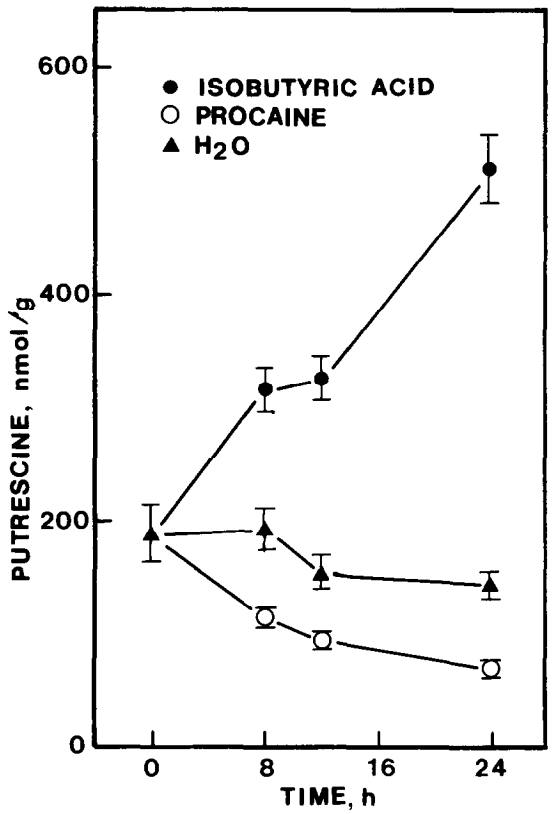

Fig. 4. Changes with time in the level of putrescine in detached rice leaves treated with isobutyric acid or procaine. Detached rice leaves treated with either water, 5 mM isobutyric acid or 5 mM procaine in darkness. Putrescine was determined at the times indicated. Bars indicate standard errors (n = 3).

possibility will not be firmly established until change of the cytosolic pH is shown to occur.

Acknowledgement

This work was supported financially by the National Science Council of the Republic of China (NSC 82-0409-B 002-030).

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