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Ammonium, calcium, and leaf senescence in rice

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Abstract

The possible involvement of calcium in the regulation of ammonium-promoted senescence of detached rice leaves was investigated. Calcium effectively reduced ammonium-promoted senescence of detached rice leaves. The effect of ammonium on the senescence was also significantly reduced by the calcium ionophore A23187. Ammonium-promoted senescence of detached rice leaves may be mediated through blocking the entrance of calcium ions into the cytosol.

Abbreviations: MSO = methionine sulfoximine

1. Introduction

Ammonium is considered to be toxic to plant cells [5]. Various investigatiations have been able to show that the accumulation of ammonium in leaves during senescence [14, 19]. Recently, we reported that ammonium promoted senescence of detached rice leaves [2]. We also demonstrated that ammonium accumulation is associated with dark-induced senescence of detached rice leaves [2]. It has been shown that calcium was effective in retarding senescence of detached rice leaves in darkness and the calcium ionophore A23187, believed to raise the cytosolic level of calcium ions, was quite effective in reducing senescence of detached rice leaves in darkness [7]. It appears that calcium may also interact with ammonium in the regulation of senescence of detached rice leaves. In this study, the possible involvement of calcium in the regulation of ammonium-promoted senescence of detached rice leaves was investigated.

2. Materials and methods

Rice (*Oryza sativa* cv. Taichung Native 1) was cultured as previously described [10]. The apical 3-cm segments excised from the third leaves of 12-d-old seedlings were used. A group of 10 segments was floated in a Petri dish containing 10 mL of test solutions. Incubation was carried out at 27 °C in darkness.

For protein determination, leaf segments were homogenized in 50 mM sodium phosphate buffer (pH 6.8). The extracts were centrituged at 17,600 g for 20 mm, and the supernatant liquids were used for determination of protein by the method of Bradford [1]. Protein level was expressed as mg g^{-1} fresh weight.

For ammonium determination, leaf segments were homogenized in 0.3 mM sulphuric acid (pH 3.5). The homogenate was centrifuged for 10 min at 39,000 g and the supernatant was used for determination of ammonium by the method described previously [12]. Ammonium level was expressed as μmol g^{-1} fresh weight.

All experiments were repeated three times and within each experiments, treatments were replicated 4 times.

3. Results and discussion

The senescence of detached rice leaves is characterized by a decrease in chlorophyll and protein levels [10]. The decrease in chlorophyll and protein levels

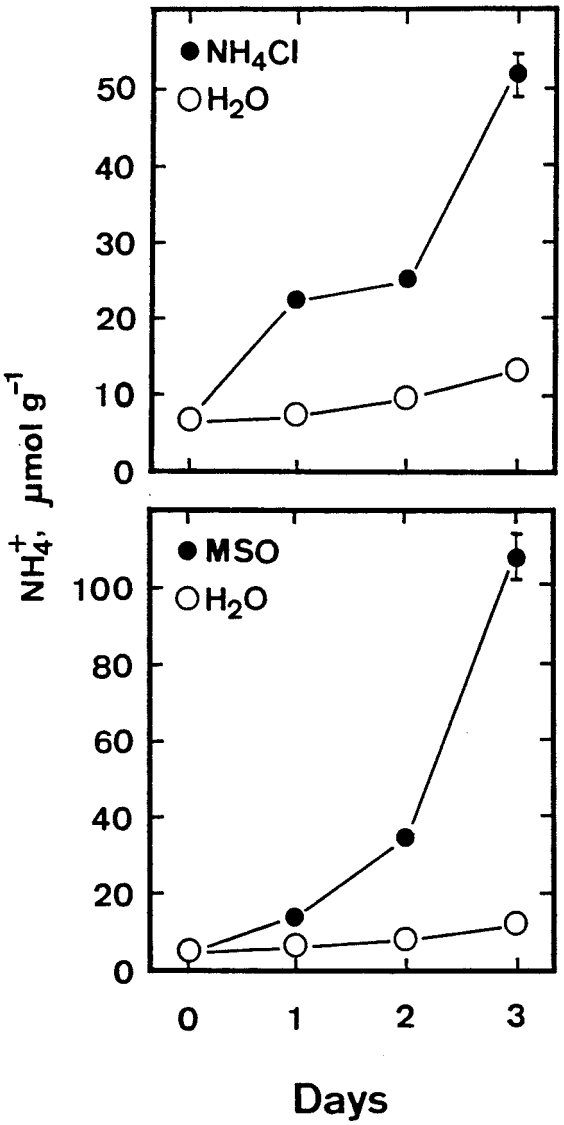

Figure 1. Time courses of ammonium level in detached rice leaves treated with water or methionine sulfoximine (MSO, 0.5 mM) or NH_4Cl (100 mM). Bars indicate SE (n = 4). Only those SE larger than symbol size are shown.

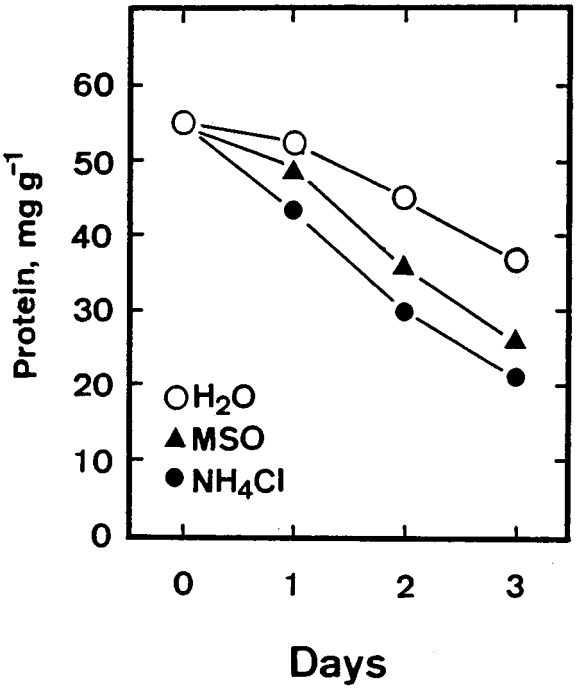

Figure 2. Time courses of protein level in detached rice leaves treated with water or MSO or NH_4Cl in darkness. The concentration of MSO and NH_4Cl was 0.5 mM and 100 mM, respectively. Bars indicate SE (n = 4). Only those SE larger than symbol size are shown.

has been the principal criterion of leaf senescence for a large number of workers [18]. The decrease in protein level in detached rice leaves during senescence precedes that in chlorophyll level [10]. For this reason, the senescence of detached rice leaves was followed by measuring the decrease of protein in the present study.

Methionine sulfoximine (MSO) is a structural analogue of glutamate, and serves as an irreversible inhibitor of glutamine synthetase [15, 16]. There is evidence that application of MSO results in accumulation of ammonium [13]. It is expected that application of MSO and NH_4Cl would increase endogenous ammonium levels in detached rice leaves. As indicated in Figure 1, this is indeed the case.

Figure 2 shows the time courses of protein breakdown of detached rice leaves floating on water, NH_4Cl (100 mM), or MSO (0.5 mM) in the dark. It is clear that NH_4Cl or MSO significantly promotes the senescence of detached rice leaves. These results are in agreement with our recent report [2].

Previous studies of various systems have indicated that calcium plays an important role in regulating leaf senescence [3, 4, 6–9, 11]. Recently, Huang et al. [6] provide direct evidence for the elevation of cytosolic calcium ions during senescence process in detached parsley leaves. However, our indirect evidence indicated that elevation of cytosolic calcium ions is required to retard the senescence of detached rice leaves [3, 7]. Clearly, the effect of calcium on rice leaf senescence is different from that on detached parsley leaves. This discrepancy may attribute to different plant leaves used.

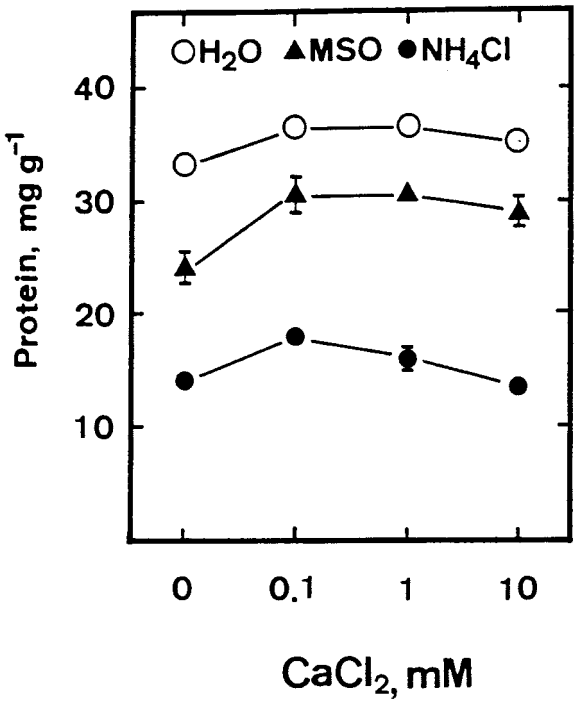

Figure 3. Effect of $CaCl_2$ on MSO- and NH_4Cl-promoted senescence of detached rice leaves. Segments of rice leaves were incubated in various concentrations of $CaCl_2$ in the presence of MSO (0.5 mM) or NH_4Cl (100 mM). Protein was determined after 3 days in darkness. Bars indicate SE (n = 4). Only those SE larger than symbol size are shown.

To see whether calcium interacts with ammonium in the control of rice leaf senescence, the effect of calcium on protein level in the presence of NH_4Cl or MSO was tested and the results are presented in Figure 3. Calcium significantly increased protein level in the presence of NH_4Cl or MSO.

To characterize further the role of calcium in the regulation of senescence of detached rice leaves, the calcium ionophore A23187 was applied to detached rice leaves. The results shown in Table 1 indicate that the senescence promoting effect of NH_4Cl or MSO was significantly reduced by A23187. The effect of A23187 in the absence of $CaCl_2$ was similar to that in the presence of $CaCl_2$.

The results of the present investigation provide further evidence to support our earlier conclusion that the cytosolic calcium ions are important in the regulation of senescence of detached rice leaves [3, 7]. Our experiments also suggest that ammonium-promoted senescence of detached rice leaves may be mediated through blocking calcium ions into cytosol.

Table 1. Effect of MSO (0.5 mM) or NH_4Cl (100 mM) alone and MSO or NH_4Cl in presence of A23187 (10 μM) or $CaCl_2$ (0.1 mM) on protein level of detached rice leaves in darkness

Treatment	Protein, mg g^{-1}
Control	27.73 ± 1.15
MSO	23.90 ± 0.43
MSO + A23187	26.36 ± 0.55
MSO + A23187 + $CaCl_2$	27.09 ± 0.22
NH_4Cl	22.44 ± 0.76
NH_4Cl + A23187	26.75 ± 0.44
NH4Cl + A23187 + $CaCl_2$	26.82 ± 0.71

All solutions included 5% ethanol. Protein level was determined after 3 days in darkness. Means ± SE (n = 4)

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