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Ammonium in relation to proline accumulation in detached rice leaves

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Abstract

Ammonium accumulation in relation to proline accumulation in detached rice leaves under stress conditions was investigated. Ammonium accumulation in dark-treated detached rice leaves preceded proline accumulation. Ammonium accumulation caused by water stress coincided closely with proline accumulation in detached rice leaves. Exogenous NH_4Cl and methionine sulfoximine (MSO), which caused an accumulation of ammonium in detached rice leaves, increased proline content. It was found that proline in NH_4Cl- or MSO-treated rice leaves is less utilized than in water-treated rice leaves (controls). These results are in agreement with the observation that a decrease in proline utilization contributes to the accumulation of proline in dark-treated and water stressed rice leaves. Although ammonium content increased in Cd- and Cu-treated rice leaves, the increase in ammonium content was only observed after the increase in proline content.

Abbreviations: MSO – methionine sulfoximine; WS – water stress

1. Introduction

Proline has been shown to accumulate in plants subjected to water stress and exposed to excess Cd and excess Cu [1, 2, 9, 12, 26, 27, 29, 32]. Previously, we have reported that proline accumulated in detached rice leaves during dark-induced senescence [10, 31].

The ammonium ion is a central intermediate in the metabolism of nitrogen in plants. Glutamine synthetase plays a crucial role in the assimilation of ammonium [16]. It has been shown that water stress results in a decline in the activity of glutamine synthetase in leaves [4, 18]. Glutamine synthetase activity is also known to decrease during dark-induced senescence of leaves [7, 13, 20, 28]. We have demonstrated that excess Cu decreased the activity of glutamine synthetase in detached rice leaves [7]. A decline in glutamine synthetase activity in leaves during water stress, dark-induced senescence or when exposed to heavy metals may result, at least in part, in an accumulation of ammonium in leaves. In fact, ammonium has been shown to accumulate in leaves subjected to water stress, when exposed to excess Cu, and during dark-induced senescence [7, 8, 18, 22, 30].

Accumulation of putrescine has been reported in response to environmental stresses [6]. Several lines of evidence suggest that putrescine biosynthesis is linked to the assimilation of ammonium nutrition in both stressed and nonstressed plant tissues. Le Rudulier and Goas [15] showed that ammonium nutrition produced higher tissue putrescine content than did nitrate feeding. Similarly, Klein et al. [14] reported that K^+-deficient peas utilizing ammonium as a nitrogen source accumulated putrescine to a greater extent than those maintained on nitrate. Rabe and Lovatt [24] have demonstrated that increased ammonium content in PO_4-deficient citrus are accompanied by increased arginine biosynthesis. Rabe [23] suggested that putrescine and arginine accumulating during environmental stress conditions might serve as an ammonium detoxification mechanism. By extension, it seems likely that stress-induced accumulation of proline could represent a manifestation of such an ammonium detoxification mechanism. To understand the metabolic

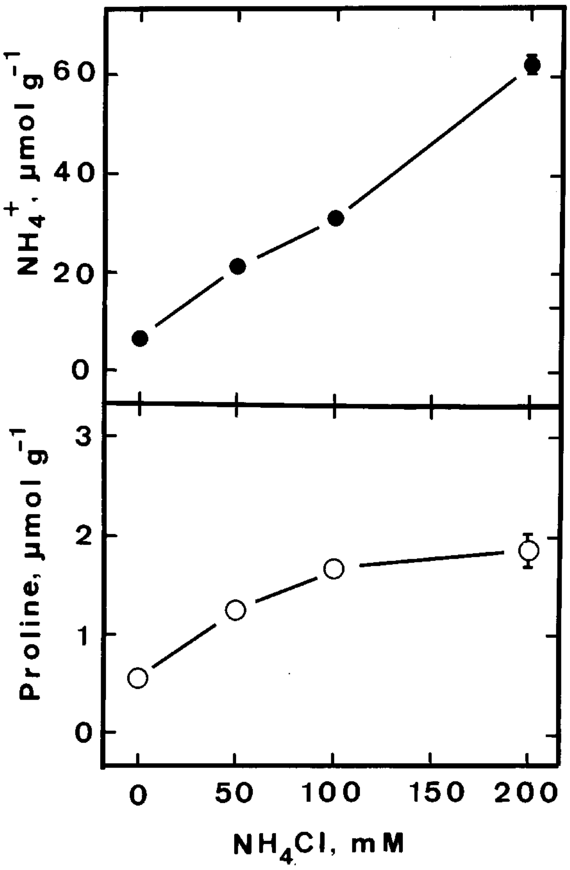

Figure 1. Influence of NH_4Cl on ammonium and proline contents in detached rice leaves. All measurements were made 12 h after treatment in the light. Vertical bars represent standard errors (n = 4).

and cellular phenomena that lead to proline accumulation, in detached rice leaves induced by water stress, heavy metals and during dark-induced senescence, we have tried to determine the relation, if any, between ammonium and proline accumulations. This paper reports more detailed time-course measurements along with some dose-response experiments, designed to describe the relation between ammonium and proline accumulations in detached rice leaves.

2. Materials and methods

Rice (*Oryza sativa* cv. Taichung Native 1) was cultured on a stainless net floating on half-stength Johnson's modified nutrient solution (pH 4.2) in a 500 ml beaker [11]. The nutrient solution was replaced every three days. Rice seedlings were grown for 12 d in a greenhouse, where natural light was provided and the temperature was controlled at 30 °C during the day and at 25 °C at night. 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 or water (controls). Incubation was carried out at 27 °C in light (40 μmol quanta m^{-2} s^{-1}) or in darkness. Water stress was applied by a previously described method [12]. Briefly, leaf segments were exposed to the vapour above a solution of 0.5 M NaCl to decrease air humidity.

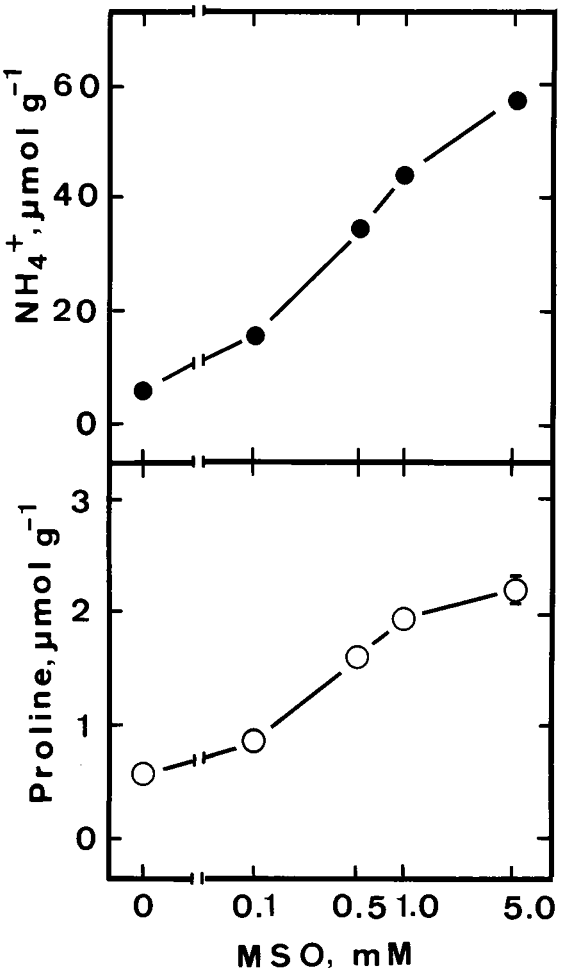

Figure 2. Influence of MSO on ammonium and proline contents in detached rice leaves. All measurements were made 12 h after treatment in the light. Vertical bars represent standard errors (n = 4).

Proline was extracted and its concentration determined by the method of Bates et al. [3]. Leaf segments were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged. The supernatant was treated with acetic acid and acid ninhydrin, boiled for

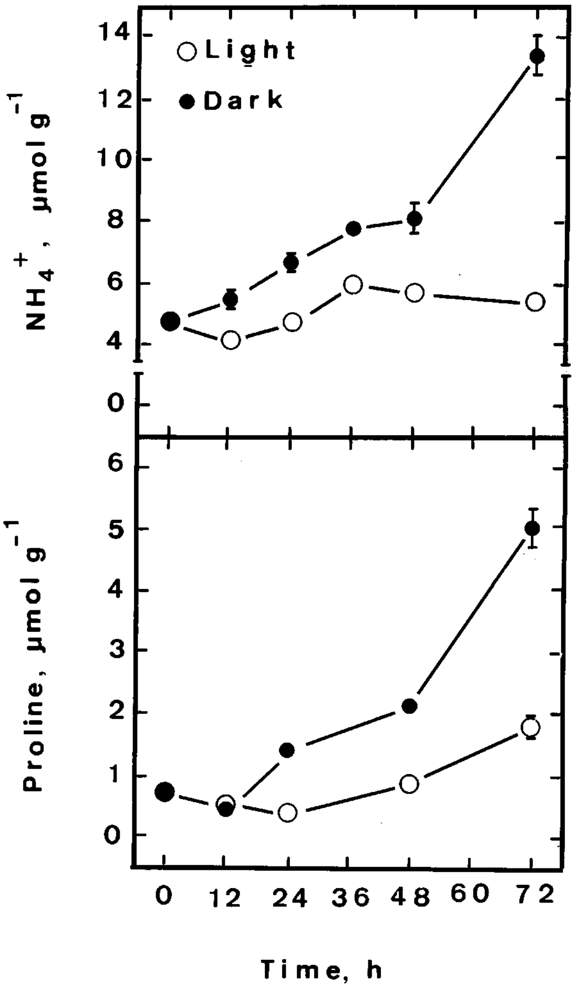

Figure 3. Time courses of proline and ammonium contents in detached rice leaves incubated in distilled water under dark and light conditions. Vertical bars represent standard errors (n = 4).

1 h and then absorbance at 520 nm was determined. Amounts of proline are expressed as μmol g^{-1} initial fresh weight.

Ammonium was extracted by homogenizing leaf segments 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 as described previously [17]. Ammonium content was expressed as μmol g^{-1} initial fresh weight.

3. Results and discussion

Figure 1 shows the effect of NH_4Cl on ammonium and proline contents in detached rice leaves in the light. It is clear that the increase in the ammonium content in NH_4Cl-treated detached rice leaves was associated with the increase in proline content. MSO is a structural analogue of glutamate, and serves as an irreversible inhibitor of glutamine synthetase [25]. There is evidence that the addition of MSO results in an accumulation of ammonium [5, 21]. To characterize further the relationship between ammonium and proline contents in detached rice leaves, detached rice leaves were incubated in the presence of various concentrations of MSO. As indicated in Figure 2, MSO increased endogenous ammonium and proline contents.

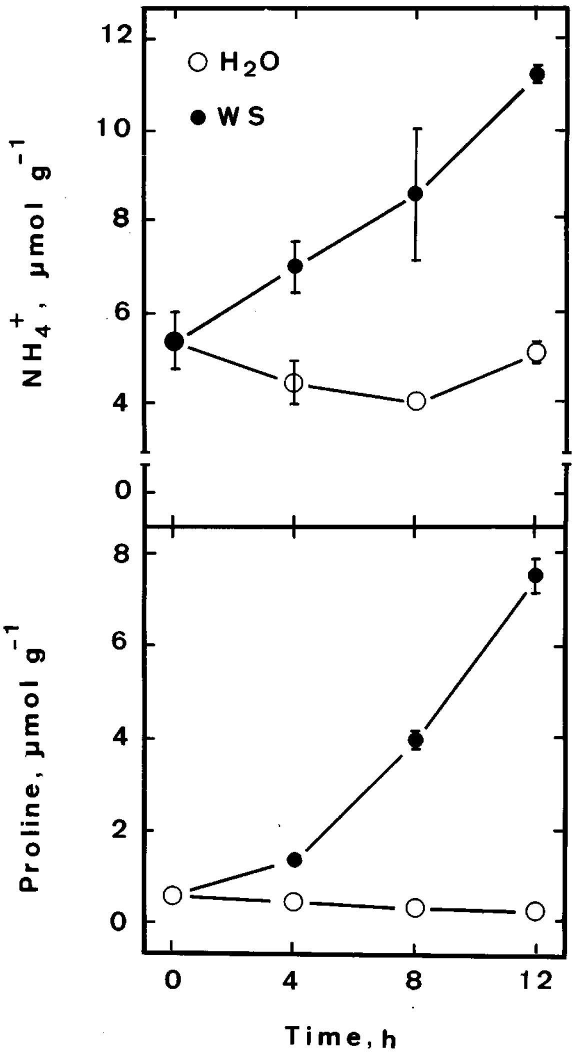

Figure 4. Time courses of proline and ammonium contents in detached rice leaves incubated in distilled water and water stress (WS) conditions. Vertical bars represent standard errors (n = 4).

Table 1. Proline content in ornithine-pretreated detached rice leaves incubated in distilled water, NH_4Cl and MSO

Treatment	Proline, μmol g^{-1}
Orn, 3h	18.7 ± 1.3
Orn, 3h → H_2O, 8h	5.7 ± 0.4
Orn, 3h → NH_4Cl, 8h	10.6 ± 0.7
Orn, 3h → MSO, 8h	9.1 ± 0.7

Detached rice leaves were pretreated with 50 mM ornithine (Orn) for 3 h in the light and then incubated in distilled water, NH_4Cl (200 mM), or MSO (1 mM) for 8 h in the light. Means ± SE (n = 4).

Figure 3 shows the change of proline content in detached rice leaves incubated in distilled water in darkness and in the light. In darkness, the amount of proline in detached rice leaves did not increase until 12 h after incubation, but increased about 2-, 2.5- and 6-fold at 12, 48, and 72 h, respectively, which confirmed our previous results [10, 31]. However, the amount of proline in detached rice leaves, incubated in the light remained relatively unchanged during 48 h, but subsequently increased slightly. In detached rice leaves, it is apparent that proline accumulates more in darkness than in the light. It is expected that the ammonium content is greater in darkness than in the light. As indicated in Figure 3, this is indeed the case. An increase in the ammonium content of dark-treated detached rice leaves was found to precede the increase in proline contents.

It has long been recognized that water stress is effective in increasing proline content in detached rice leaves [12]. The effect of water stress on proline content in relation to ammonium content is presented in Figure 4. Water stress effectively increased proline and ammonium content.

Recently, we have shown that a decrease in proline utilization contributes to the accumulation of proline in dark-treated and water stressed rice leaves [33]. If ammonium accumulation is indeed important in regulating dark- and water stress-induced proline accumulation in detached rice leaves, NH_4Cl or MSO is expected to decrease proline utilization. To test this possibility, detached rice leaves were pretreated with 50 mM ornithine for 3 h to increase the endogenous proline content and then transferred to NH_4Cl or MSO for 8 h. Proline content was then determined. As indicated in Table 1, proline content in NH_4Cl- or MSO-treated rice leaves was greater than in water-treated control rice leaves, suggesting that proline in NH_4Cl- or MSO-treated rice leaves is less utilized than in controls. These results supported further that ammonium accumulation in dark-treated and water stressed rice leaves is associated with proline accumulation.

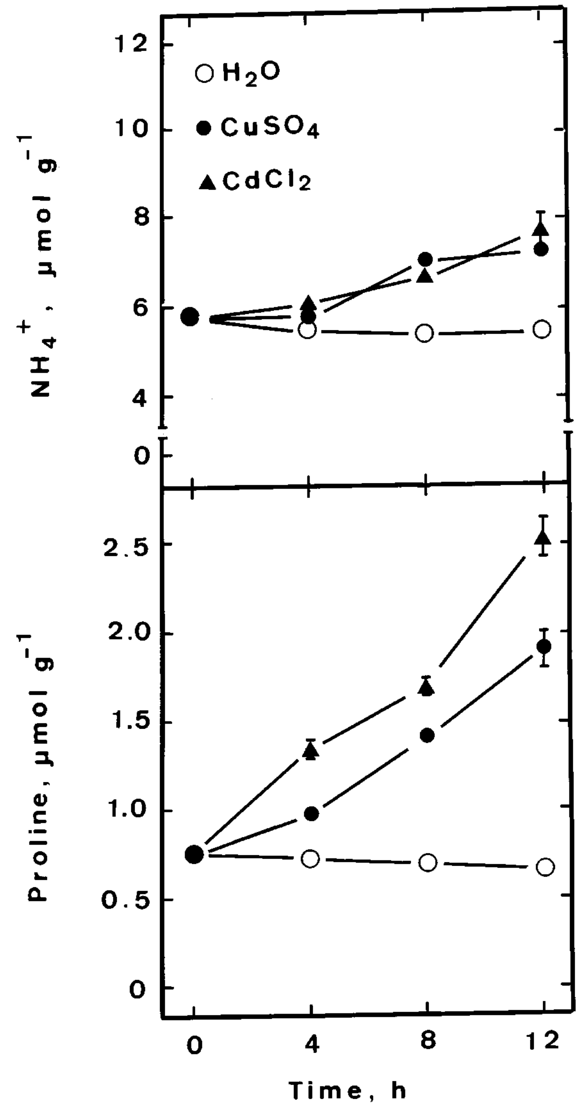

Figure 5. Time courses of proline and ammonium contents in detached rice leaves incubated in distilled water, $CdCl_2$ (5 mM) and $CuSO_4$ (10 mM). Vertical bars represent standard errors (n = 4).

Proline has been shown to accumulate in plants exposed to excess Cd or Cu [2, 9, 26, 27, 32]. In detached rice leaves, treatment of Cd or Cu also resulted in an accumulation of proline (Figure 5), However, proline accumulation in Cd- and Cu-treated detached rice leaves preceded ammonium accumulation (Figure 5). It is apparent that ammonium accumulation is not involved in Cd- and Cu-induced proline accumulation in detached rice leaves.

Results from studies conducted in varieties of citrus which were tolerant or susceptible to phosphorus deficiency and cold treatment showed increased ammonium content and increased rate of arginine biosynthesis in leaves exposed to stress [19, 23]. Susceptible plants had lower rates of arginine biosynthesis and higher ammonium content; tolerant plants had greater rates of arginine biosynthesis and lower ammonium content. These data suggest that there is a positive correlation between detoxification of ammonium, arginine biosynthesis and tolerance to several types of environmental stress. Similar findings were reported for studies conducted on alfalfa suspension cultures subjected to osmotic shock as well as cucurbits exposed to salinity stress [19]. In the present investigation, we are able to show that ammonium accumulation in dark-treated and water stressed rice leaves is associated with proline accumulation. Future work should be focused on the correlation between conversion of ammonium into proline and tolerance to dark-induced senescence and water stressed in rice leaves.

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