

BRIEF COMMUNICATION

Proline, ornithine, arginine and glutamic acid contents in detached rice leaves

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Abstract

The effects of water stress on the contents of proline, ornithine, arginine and glutamic acid in detached rice leaves were examined. In water stressed leaves, the content of proline was elevated to a content approximately 8-, 14- and 17-fold higher than in control leaves after 4, 8 and 12 h, respectively. We also observed that ornithine and arginine contents were much higher under water stress than in control leaves. However, the content of glutamic acid in water stressed leaves was higher after 4 and 8 h and lower after 12 h than that in control leaves.

Additional key words: amino acids, *Oryza sativa*, protein, water stress.

Proline often accumulates in plants subjected to water stress (e.g. Munns *et al.* 1979, Kao 1981, Venckamp and Kost 1988, Mattioni *et al.* 1997, Yoshiba *et al.* 1997). It has been shown that pyrroline-5-carboxylate synthetase, which acts during the biosynthesis of proline, and proline dehydrogenase, which acts during the degradation of proline, appear to be the rate-limiting factors responsible for proline accumulation in water-stressed plant tissues (Yoshiba *et al.* 1997). Recently, we have shown that a decrease in proline utilization might contribute to water stressed-induced proline accumulation in detached rice leaves (Yang *et al.* 2000). Very little, if any, is known whether the contents of three amino acids (glutamic acid, ornithine and arginine) involved in the proline biosynthetic pathway are limiting factors for proline accumulation in plant tissues. The main question has been addressed in this paper: Is the concentration of glutamic acid, ornithine and arginine related to proline accumulation in detached rice leaves caused by water stress?

Rice (*Oryza sativa* L., cv. Taichung Native 1) seedlings were cultured as previously described (Yang *et al.* 2000). The apical 3-cm segments were excised from the

third leaves of 12-d-old seedlings. Groups of twenty leaf segments were exposed to slightly decreased relative humidity (RH) above a solution of 0.5 M NaCl. Similar segments of leaves were floated on 20 cm³ of distilled water in a Petri dish to serve as control. Water stress was judged by determination of relative water content (RWC) according to Mukherjee and Choudhuri (1983). All samples were incubated at 27 °C and irradiance 40 µmol m⁻² s⁻¹. For determination of glutamic acid, ornithine, arginine, proline, and total amino acids, leaf samples were extracted with boiling 80 % ethanol for 15 min, when the leaves were totally bleached. The extract was reduced to about 1 cm³, the volume was adjusted to 2 cm³ with distilled water and centrifuged at 1 000 g for 90 s. The clear supernatant liquid was used directly for amino acid analysis (amino acid analyzer, Beckman 6300, Palo Alto, USA). For protein determination, leaf segments were homogenized in 50 mM sodium phosphate buffer (pH 6.8). The homogenate was centrifuged at 17 600 g for 20 min, and the supernatants were used for determination of protein by the method of Bradford (1976). All data were expressed on the basis of initial fresh mass.

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Abbreviations: f. m. - fresh mass; RH - relative humidity; RWC - relative water content.

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

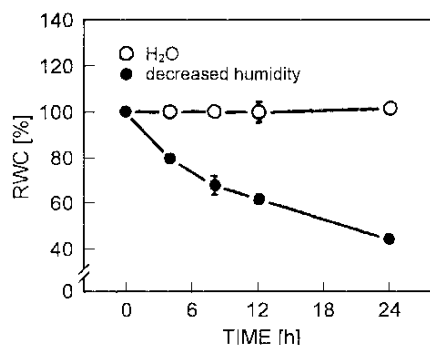


Fig 1. Relative water content (RWC) in detached rice leaves floating on water (H₂O) or exposed to slightly decreased air humidity above 0.5 M NaCl (WS) for 4, 8 and 12 h. Vertical bars represent SE ($n = 4$). Only those SE larger than symbol size are shown.

Table 1. Contents of proline, glutamic acid, ornithine, arginine [nmol g⁻¹(f.m.)], protein [mg g⁻¹(f.m.)], and total amino acids [μ mol g⁻¹(f.m.)] in detached rice leaves floating on water (H₂O) or exposed to decreased air humidity for 4, 8 and 12 h (WS).

	0 h		4 h		8 h		12 h		
			H ₂ O	WS	H ₂ O	WS	H ₂ O	WS	
Proline	162.8±	5.2	135.8±	3.6	1028.2±	40.9	275.8±	4.0	3963.2± 96.3
Glutamic acid	5258.8±	273.5	3742.0±	246.0	7012.7±	560.3	5465.7±	45.1	8261.1±361.7
Arginine	376.6±	18.9	194.6±	4.1	321.2±	20.7	136.1±	3.8	1208.8± 54.0
Ornithine	12.9±	0.4	6.4±	0.4	18.6±	4.1	9.1±	0.1	24.7± 2.4
Protein	58.2±	0.5	58.3±	0.7	49.7±	1.2	57.8±	2.6	44.3± 0.8
Amino acids	15.6±	0.6	3.5±	0.2	32.7±	2.5	20.5±	0.1	62.5± 2.7
									23.8± 0.4
									63.1± 2.6

It is well established that glutamic acid, ornithine and arginine can all contribute to the accumulation of proline in plant tissues (Chiang and Dandekar 1995). Ornithine and arginine contents in detached rice leaves under water stress were much higher than those in control leaves (Table 1). However, glutamic acid content in detached rice leaves exposed to water stress was higher after 4 and 8 h and lower after 12 h than that in control leaves (Table 1). These observations suggest that higher contents of endogenous ornithine and arginine are associated with water stress-induced proline accumulation in detached rice leaves. Arginine can contribute to proline biosynthesis, and the pathway from arginine proceeds via

Similar results and identical trends were obtained in all experiments. The data reported here are from a single experiment.

Relative water content (RWC) of leaf segments exposed above a 0.5 M NaCl under light decreased considerably during 24-h treatment (Fig. 1), indicating that the treatment indeed cause water stress.

In control leaves, proline content decreased after 4-h treatment and then increased slightly (Table 1). Proline content in detached rice leaves exposed to the decreased air humidity (water stressed, WS) was increased to a contents about 8-, 14- and 17-fold higher than those in control leaves at 4, 8 and 12 h, respectively (Table 1).

The decline in protein content during 12-h incubation was faster in WS than in control leaves (Table 1). Therefore, protein degradation might contribute to the water stress-induced proline accumulation. This conclusion is supported further by the observation that total amino acid content in detached rice leaves was higher under water stress than in controls (Table 1).

ornithine as a result of catalytic activity of arginase (Brown and Fowden 1966). Since ornithine is more effective than arginine in increasing proline content in detached rice leaves (Yang *et al.* 2000), the contribution of ornithine pathway plays more determinant role in proline accumulation in detached rice leaves. In higher plants, it has been demonstrated that ornithine- δ -aminotransferase participates in proline biosynthesis via ornithine pathway (Delauney *et al.* 1993, Roosens *et al.* 1988). However, our recent work showed that ornithine- δ -aminotransferase contributes little, if any, to proline accumulation in detached rice leaves under water stress condition (Yang and Kao 1999).

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