BIOLOGIA PLANTARUM 49 (3): 405-409, 2005

Effect of aluminium on endosperm reserve mobilization in germinating rice grains

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Abstract

The effect of $AlCl_3$ on endosperm reserve mobilization of rice grains or dehulled rice grains during germination was investigated. $AlCl_3$ had no effect on grain fresh and dry masses, protein and starch contents, and α-amylase and protease activities in endosperm of germinating rice grains. However, when dehulled rice grains were treated with $AlCl_3$, $AlCl_3$ inhibited the decrease in fresh mass, dry mass, and starch and protein contents, and the increase in α-amylase and protease activities in endosperm. Evidence is provided to show that the hull is a barrier against influx of Al to endosperm.

Additional key words: α-amylase, *Oryza sativa*, protease.

Introduction

Aluminium, the third most abundant element in the earth crust, has been recognized as an inhibitor factor for most crop production on acid soils which make up a large proportion of arable land in the tropical and subtropical areas. The most dramatic symptom of Al phytotoxicity is the reduction of root growth (Matsumoto 2000). However, the mechanisms of the root growth inhibition by Al have not been fully elucidated.

Cereals, such as rice and barley, store starch and protein in their endosperm cells. Starch and protein are degraded mainly by α-amylase and protease, respectively, and are mobilized to supply sources of carbon, amino acids and energy for the growth of embryos (Bewley 1997, Pritchard *et al.* 2002). Al inhibits growth of rice roots (Nishizawa 1995). Thus, it is not unreasonable to speculate that Al may inhibit endosperm mobilization in germinating rice grains. In a recent report, Kataoka *et al.* (2003) demonstrated that aluminium treatment decreased apoplast protein in soybean root tips.

In this paper, we report the effect of $AlCl_3$ on the changes in the contents of starch and protein and the activities of α-amylase and protease in endosperm of germinating dehulled rice grains and rice grains.

Materials and methods

Dehulled rice (*Oryza sativa* L., cv. Taichung Native 1) grains or rice grains were surface-sterilised in 2.5 % (v/v) sodium hypochlorite for 15 min, washed in distilled water, sown on moistened filter paper in a Petri dish (20 cm), and incubated at 37 °C. After 24-h incubation, seedlings with 2 mm shoots were selected and transferred to Petri dishes containing two sheets of filter paper moistened with 10 cm^3 of distilled water (pH 4.0) or 0.5 mM $AlCl_3$ (pH 4.0) and allow to grow at 27 °C in the dark. Distilled water or $AlCl_3$ solution was changed every 3 d. Each Petri dish contained 20 germinated dehulled rice grains or rice grains. Each treatment replicated 4 times.

Starch in endosperms was extracted twice with hot ethanol (80 %, v/v). The tissue residues were suspended in 2 mM sodium phosphate buffer (pH 6.9) containing 6 mM NaCl, and were boiled for 15 min to gelatinize the

Received 3 October 2003, *accepted* 10 November 2004.
Abbreviations: d.m. - dry mass, f.m. - fresh mass.
Acknowledgements: This work was supported by the National Science Council of the Republic of China, grant NSC92-2313-B-002-001.
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starch. Crude boiled homogenates were then used to determine starch level according to the method described previously (Hurng and Kao 1993). Briefly, crude boiled homogenates containing starch were digested for 16 h at 37 °C with 25 units of *Bacillus* α-amylase (*Sigma-Aldrich*, St. Louis, Missouri, USA) in sodium phosphate buffer. Blanks were comprised of sample aliquots and heat inactivated α-amylase. Maltose produced from starch was determined by the dinitrosalicylic acid (Hurng and Kao 1993). Starch level is expressed as mg maltose equivalent per endosperm.

For α-amylase extraction, endosperm was homogenised in prechilled mortar and pestle with 0.2 M sodium acetate buffer (pH 5.4) containing 3 mM $CaCl_2$ at 4 °C. The homogenate was centrifuged at 12 000 *g* for 20 min at 4 °C. Crude extract was used to determine α-amylase activities based on the method developed by Rinderknecht *et al*. (1967) using starch azure as substrate. The absorbance was measured by spectrophotometer (model *U 2000*, *Hitachi*, Tokyo, Japan) and one unit of enzyme activity is defined as ΔA_{595} min^{-1}.

For protease extraction, endosperms were homogenised in prechilled mortar and pestle with 10 mM Tris-HCl buffer (pH 7.4) containing 10 mM 2-mercaptoethanol at 4 °C. The homogenate was centrifuged at 15 000 *g* for 30 min at 4 °C. The supernatant was used both for protein and protease assay. Protease was assayed according to the method described by Sheoran and Garg (1978). Protease activity was calculated based on ΔA_{280} min^{-1}. Protein was determined by the method of Bradford (1976).

For determination of Al content, endosperms or hulls were dried at 65 °C for 48 h. Dried material was ashed at 550 °C for 20 h. Ash residue was incubated with 31 % (v/v) HNO_3 and 17.5 % (v/v) H_2O_2 at 70 °C for 12 h, and dissolved in 0.1 M HCl. Al was then quantified using an atomic absorption spectrophotometer (*Model AA-680, Shimadzu*, Kyoto, Japan)

Statistical differences between measurements on different treatment or on different times were analyzed by Duncan's multiple range test or Student's *t*-test.

Results and discussion

Grain dry mass (d.m.) decreased with duration of germination in both control as well as $AlCl_3$-treated grains (Table 1). However, no difference on d.m. was observed between H_2O- and $AlCl_3$-treated grains. Basically, during germination no change in grain fresh mass (f.m.) was observed in H_2O-treated grains. Again, $AlCl_3$ had no effect on grain f.m.

Starch is an important reserve in endosperm of rice grains. During germination, starch content in endosperm of rice grains decreased (Table 2). However, endosperm of grains treated with $AlCl_3$ had similar starch content as that treated with distilled water during germination (Table 2). Mobilization of starch in endosperm is mediated by α-amylase (Yu *et al*. 1992). Comparative study of α-amylase activity in endosperm revealed a continuous increase in the activity of enzyme during germination similarly in both control as well as $AlCl_3$-treated ones (Table 2).

Table 1. Effect of $AlCl_3$ on the changes in grain fresh mass and grain dry mass in germinating rice grains. Rice grains were incubated in water (pH 4.0) or 0.5 mM $AlCl_3$ (pH 4.0). Means ± SD, n = 40.

Time [d]	Fresh mass [mg $grain^{-1}$]		Dry mass [mg $grain^{-1}$]	
	H_2O	$AlCl_3$	H_2O	$AlCl_3$
0	34.4 ± 0.07		25.4 ± 0.19	
1	35.6 ± 0.30	35.4 ± 0.16	24.5 ± 0.22	24.7 ± 0.34
3	35.0 ± 0.06	34.9 ± 0.19	21.9 ± 0.20	22.3 ± 0.04
5	33.8 ± 0.22	33.5 ± 0.23	18.7 ± 0.18	18.7 ± 0.27

Table 2. Effect of $AlCl_3$ on the changes in starch level and α-amylase activity in endosperm of germinating rice grains. Rice grains were incubated in water (pH 4.0) or 0.5 mM $AlCl_3$ (pH 4.0). Means ± SD, n = 4.

Time [d]	Starch [mg $endosperm^{-1}$]		α-Amylase activity [U $endosperm^{-1}$]	
	H_2O	$AlCl_3$	H_2O	$AlCl_3$
0	13.3 ± 0.20		0.22 ± 0.004	
1	13.5 ± 0.45	13.8 ± 0.60	0.23 ± 0.02	0.25 ± 0.02
3	10.7 ± 0.99	11.4 ± 0.15	1.63 ± 0.02	1.61 ± 0.02
5	7.5 ± 0.53	8.2 ± 0.37	1.66 ± 0.02	1.66 ± 0.06

Endosperm of rice grains treated with $AlCl_3$ had same content of proteins as that of grains treated with distilled water during germination (Table 3). During seed germination, mobilization of endosperm reserve proteins is mediated through the action of proteases (Beevers 1968, Yomo and Varner 1973). In control, protease activity increased significantly during germination (Table 3) and $AlCl_3$ did not inhibit protease activity during germination (Table 3).

All these results seem to suggest $AlCl_3$ had no effect on endosperm mobilization during germination of rice grains. This is in contrast to our previous work, in which we reported that NaCl markedly decreased the mobilization of starch in endosperm of germinating rice grains (Lin and Kao 1995).

Rice grain is the ripened ovary, with the lemma, palea, rachilla, sterile lemmas, and the awn, if present, firmly

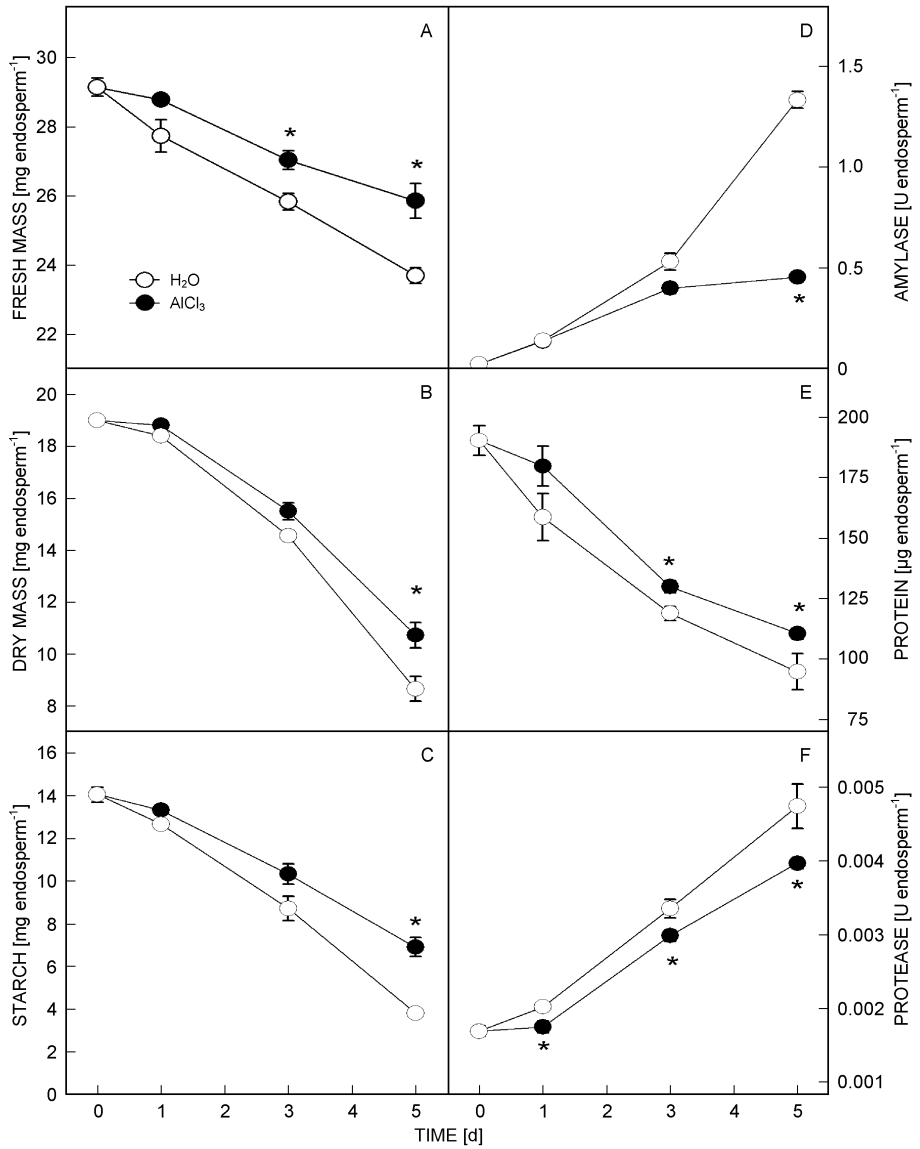

Fig. 1. Effect of $AlCl_3$ on the changes in fresh mass (f.m.) (*A*), dry mass (d.m.) (*B*), starch content (*C*), amylase activity (*D*), protein content (*E*), and protease activity (*F*) in endosperm of germinating dehulled rice grains. Dehulled rice grains were incubated in water (pH 4.0, *open circles*) or 0.5 mM $AlCl_3$ (pH 4.0, *closed circles*). Means for f.m. and d.m. calculated from 40 endosperms, whereas means for the contents of starch and protein and the activities of amylase and protease calculated from 4 replicates. *Asterisks* indicate the differences significant between H_2O and $AlCl_3$ treatments at $P < 0.05$ level calculated by Student's *t*-test.

adhered to it. The lemma and palea and their associated structures such as the sterile lemmas, rachilla, and the awn whenever present, constitute the hull or husk. It is not known whether the hull acts as a barrier against the influx of Al. To test this possibility, we determined the Al content in the hull and endosperm, respectively, of germinating rice grains treated with $AlCl_3$. Al content in the hull increased markedly with duration of germination of rice grains treated with $AlCl_3$ (Table 4). However, only slight increase in Al level was observed in the endosperm (Table 4). Al content in the endosperm of germinating dehulled rice grains treated with $AlCl_3$ was significantly higher than in controls. Thus, the fact that $AlCl_3$ did not

Table 3. Effect of $AlCl_3$ on the changes in protein level and protease activity in endosperm of germinating rice grains. Rice grains were incubated in water (pH 4.0) or 0.5 mM $AlCl_3$ (pH 4.0). Means ± SD, $n = 4$.

Time [d]	Protein [µg endosperm^{-1}]		Protease activity [U endosperm^{-1}]	
	H_2O	$AlCl_3$	H_2O	$AlCl_3$
0	153 ± 13		0.0018 ± 0.00008	
1	130 ± 23	126 ± 25	0.0021 ± 0.00007	0.0020 ± 0.00013
3	78 ± 21	96 ± 13	0.0043 ± 0.00013	0.0041 ± 0.00010
5	80 ± 17	74 ± 4	0.0048 ± 0.00012	0.0047 ± 0.00009

Table 4. Effect of $AlCl_3$ on the changes in Al level in endosperm and hull of germinating rice grains and in endosperm of germinating dehulled rice grains. Rice grains or dehulled rice grains were incubated in water (pH 4.0) or 0.5 mM $AlCl_3$ (pH 4.0). Mean ± SD, n = 4. *Asterisks* indicated that values are significantly different between H_2O and $AlCl_3$ treatments at $P < 0.05$ level determined by Student's t-test.

Time [d]	Rice grains				Dehulled grains	
	Al in hull [μg g^{-1}(d.m.)]		Al in endosperm [μg g^{-1}(d.m.)]		Al in endosperm [μg g^{-1}(d.m.)]	
	H_2O	$AlCl_3$	H_2O	$AlCl_3$	H_2O	$AlCl_3$
0	3.82 ± 0.48		2.99 ± 0.33		2.33 ± 0.20	
1	2.91 ± 0.70	21.85 ± 1.67*	3.73 ± 0.51	5.43 ± 0.81	2.67 ± 0.10	6.64 ± 0.18*
3	2.72 ± 0.51	25.78 ± 2.76*	4.00 ± 0.59	6.34 ± 1.15	2.68 ± 0.10	19.82 ± 1.21*
5	2.84 ± 0.60	27.85 ± 1.50*	4.29 ± 0.45	7.53 ± 1.47*	3.23 ± 0.32	32.13 ± 1.87*

affect reserve mobilization in endosperm of germinating rice grains is possibly attributable to the less amount of Al in the endosperm. If this suggestion is correct, then $AlCl_3$ is expected to inhibit endosperm mobilization in germinating dehulled rice grains. When dehulled rice grains were treated with $AlCl_3$, it was found that $AlCl_3$ inhibited the decrease in endosperm d.m. and f.m. (Fig. 1*A*,*B*), the decrease in starch and protein contents (Fig. 1*C*,*E*), and the increase in α-amylase and protease activities (Fig. 1*D*,*F*).

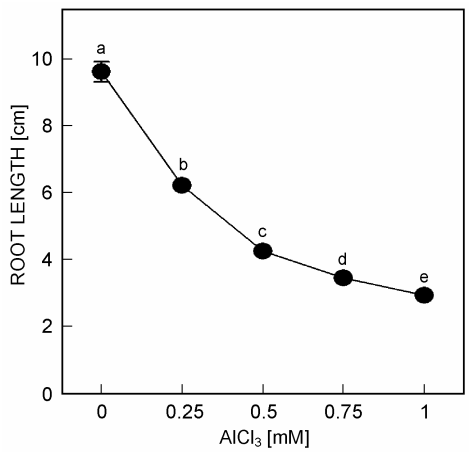

Fig. 2. Effect of $AlCl_3$ on root growth of rice seedlings. Germinating rice grains were treated with different concentrations of $AlCl_3$ (pH 4.0) for 24 h. Means with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

Al is known to inhibit root growth (Nishizawa 1995). We also show that Al inhibits root growth of rice during germination of rice grains (Fig. 2). Mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrates for the proper functioning of different metabolic processes that are essential to growth of shoot and roots (Bewley 1997, Pritchard *et al*. 2002). $AlCl_3$ has no effect on endosperm mobilization in germinating rice grains, thus, the inhibition of root growth by Al is unlikely to be attributable to lack of products of endosperm mobilization. Because the addition of sucrose, L-glutamine, and L-asparagine did not improve the root growth of seedlings in $AlCl_3$ medium (Table 5), the translocation of sucrose and amino acids from endosperm into root is unlikely inhibited by $AlCl_3$.

Table 5. Changes in root length of rice seedlings with different treatments. For all treatments, 0.25 mg dm^{-3} chlorophenicol was added to prevent bacterial growth. Root length was measured 12 h after treatment. Means ± SD, n = 4. Means with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

$AlCl_3$ [mM]	Addition	Root length [cm]
0.0	0	3.73 ± 0.08^a
0.5	0	3.03 ± 0.08^b
0.5	15 mM sucrose	3.03 ± 0.03^b
0.5	30 mM sucrose	3.03 ± 0.03^b
0.5	45 mM sucrose	3.10 ± 0.05^b
0.0	0	3.34 ± 0.04^a
0.5	0	2.59 ± 0.05^b
0.5	0.5 mM L-asparagine	2.56 ± 0.03^b
0.5	1.0 mM L-asparagine	2.70 ± 0.06^b
0.5	3.0 mM L-asparagine	2.73 ± 0.10^b
0.5	5.0 mM L-asparagine	2.66 ± 0.11^b
0.0	0	3.39 ± 0.02^a
0.5	0	2.92 ± 0.03^b
0.5	0.5 mM L-glutamine	2.92 ± 0.02^b
0.5	1.0 mM L-glutamine	2.87 ± 0.04^b
0.5	3.0 mM L-glutamine	2.86 ± 0.01^b
0.5	5.0 mM L-glutamine	2.84 ± 0.06^b

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