老化 水稻幼苗葉의 蛋白質 分解에 미치는 GA_3 와 ABA의 影響

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Effects of Gibberellic Acid and Abscisic Acid on Proteolysis of Senescing Leaves from Rice Seedlings

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The purpose of experiment : The purpose of this study was to monitor the effect of GA_3 and ABA on proteolysis of senescing leaves, and especially, their effect on KCl-enhanced proteolysis at the enzymatic level.

Materials and Methods : Five 5-cm-long leaf segments of rice seedling were placed, base down, into test tubes containing 2-ml test solutions. Five millimolar sodium phosphate, pH 7.0, served as a control, and KCl(50mM), GA_3(100 μM), and ABA(1 μM) were made up in the control buffer. Leaf segments were light-incubated at 28°C for 8 days. Five segments were harvested every other day while the rest were supplied with fresh test solutions. Crude extracts made with 5mM Sodium phosphate, pH 7.0, were passed through a PD-10 column, and the first 3.5-ml protein fraction was used as an enzyme source. In addition to hemoglobin, Rubisco, purified from 8- to 12-day-old seedling leaves, was used as a substrate. The ninhydrin-positive compounds(NPCs) were measured from the TCA-soluble supernatant for the activities of hemoglobin-degrading (H-exo) and Rubisco-degrading exoproteinases (R-exo). The difference in the amount of NPCs present in the TCA-soluble supernatant and its 12M HCl hydrolyzates was taken to be a measure of R-endo activity. The NPCs are expressed as the amount equivalent to L-leucine. Proteins and amino acids were measured. Both fresh weights of leaf samples and volumes of incubation media remained in the tubes also were measured when leaf segments were harvested every other day.

Results and Discussions: The effect of GA_3 and ABA on KCl-enhanced proteolysis of senescing leaves of rice was studied. Emphasis was given to their effects on KCl-enhanced efflux of amino acids and proteinase activity, When treated singly, GA_3 affected leaf proteolysis little, while ABA increased proteolysis, the rate of amino acid efflux, and Rubisco-degrading endoproteinase activity. An additive increase in all three parameters mentioned above was observed when leaves were treated with ABA and KCl. No such an additive effect was found when GA_3 was treated with KCl. Both GA_3 and ABA helped to alleviate the KCl-suppressed activity of Rubisco-degrading exoproteinases. The additive increase in proteolysis of rice leaves in the presence of both ABA and KCl could thus be ascribed to a further increase in the efflux of protein hydrolyzates and Rubisco-degrading endoproteinase activity. A close relationship was found between the increase in the rate of proteolysis and the decrease in water absorption of leaf segments. An increase in proteolysis was accompanied by a decrease in water absorption, and the combined treatment of ABA with KCl resulted in a further reduction of water absorption.

Table 1. Effect of GA_3 and ABA on KCl-increased proteolysis of senescing rice leaves

Compound	Treatment[1]	Incubation (days) 2	4	6	8
				g[2]	
Protein	Control	85	61	38	29
	KCl	70	38	24	16
	GA_3	88	62	38	28
	ABA	79	55	35	18
	GA_3 + KCl	72	40	30	19
	ABA + KCl	68	35	25	16
Amino acid	Control	187	306	453	458
	KCl	253	325	427	388
	GA_3	163	319	453	460
	ABA	256	359	466	425
	GA_3 + KCl	244	284	442	412
	ABA + KCl	319	413	428	408

[1] Concentrations used: GA_3, 100 μM; ABA, 1 μM; KCl, 50 mM.
[2] 100% at day 0: 239 μg proteins and 28 μg Leu eq amino acids per leaf segment.

Table 2. Cusulative amount of water absorbed by a senescing leaf segment of rice

Treatment[1]	Incubation (days) 2	4	6	8
			μl/segment	
Control	109	159	201	231
KCl	89	125	161	177
GA_3	115	160	202	223
ABA	90	135	172	209
GA_3 + KCl	87	123	153	174
ABA + KCl	69	105	135	165

[1] See Table 1 for chemical concentrations used.

Table 3. Effect of KCl, GA_3 and ABA on the amount of amino acids effluxed into the incubation media

Treatment[1]	Incubation (days) 2	4	6	8
			μg Leu eq/segment	
Control	0.56	2.20	3.28	1.46
KCl	1.24	4.84	7.70	7.36
GA_3	0.52	2.28	3.09	2.01
ABA	1.36	6.38	8.94	10.26
GA_3 + KCl	1.16	4.77	7.82	7.66
ABA + KCl	1.92	7.88	9.05	11.62

[1] See Table 1 for chemical concentrations used.

Table 4. Relative changes in R-exo and R-endo activity of rice leaves as affected by ABA and KCl

Enzyme type	Treatment[1]	Total Act. at day 2	4	6	8	Specific Act. at day 2	4	6	8
						g[2]			
Exo	Control	110	152	182	107	129	249	478	371
	KCl	93	86	82	80	133	231	344	503
	ABA	98	93	89	89	124	169	253	495
	ABA + KCl	105	105	96	83	154	298	382	522
Endo	Control	108	113	118	119	127	185	310	412
	KCl	119	130	137	139	170	341	574	874
	ABA	112	120	122	122	142	219	347	678
	ABA + KCl	127	137	145	146	187	390	577	918

[1] Concentrations used: ABA, 1 μM; KCl, 50 mM.
[2] See Figs. 2 and 3 for 100% activity at day 0 of R-exo and R-endo, respectively.

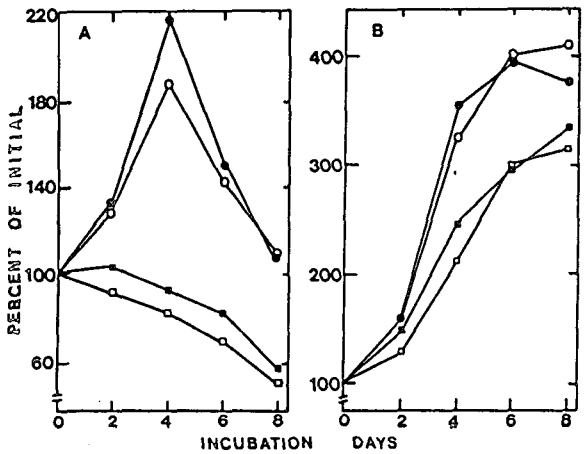

Fig. 1. Relative changes in total (A) and specific (B) activities of R-exo of rice leaves as affected by different incubation media. Leaf segments were light-incubated in the media containing 50 mM KCl (—□—), 100 μM GA_3 (—○—), and a combination of two (—●—). Five mM sodium phosphate buffer at pH 7.0 served as controls (—●—). Day 0 values (100%) of total and specific activities were, in μg Leu eq, 12.0 per leaf segment and 50.2 per mg protein, respectively.

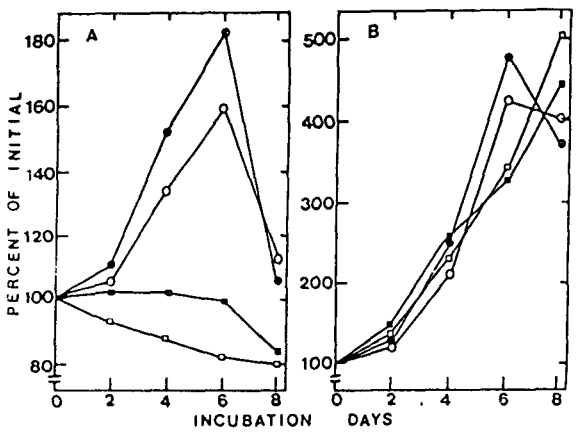

Fig. 2. Relative changes in total (A) and specific (B) activities of R-exo of senescing rice leaves as affected by different incubation media. Leaf segments were light-incubated in the media containing 50 mM KCl (--□--), 100 μM GA_3 (—○—), and a combination of two (—●—). Five mM sodium phosphate buffer at pH 7.0 served as controls (—●—). Day 0 values (100%) of total and specific activities were, in μg Leu eq, 10.6 per leaf segment and 44.4 per mg protein, respectively.

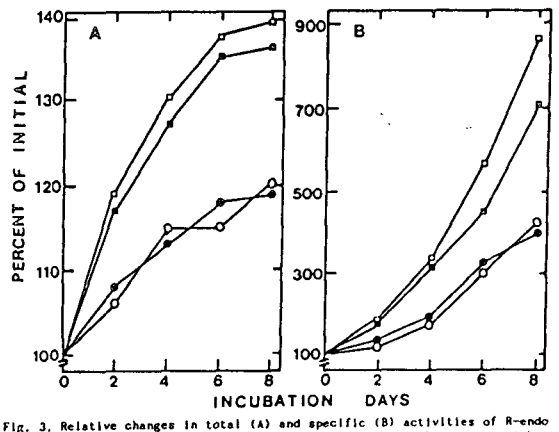

Fig. 3. Relative changes in total (A) and specific (B) activities of R-endo of senescing rice leaves as affected by GA_3 and KCl. Leaf segments, taken from the second true leaves of 16-day-old seedlings, were light-incubated in various media containing 50 mM KCl (—□—), 100 μM GA_3 (—○—), and a combination of two (—■—). Five mM sodium phosphate buffer at pH 7.0 served as controls (—●—). Purified Rubisco protein was used as a substrate. Day 0 values (100%) of total and specific activities were, in μg Leu eq, 18.3 per leaf segment and 76.6 per mg protein, respectively.