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

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老化 水稻幼苗葉의 蛋白質分解에 미치는 GA₃과 ABA의 影響

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ABSTRACT : The effect of gibberellic acid (GA_3) and abscisic acid (ABA) on KCl−enhanced proteolysis of senescing leaves of rice (*Oryza sativa* L. cv. Chilsung) 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 ribulose−1,5−bisphosphate carboxylase/oxygenase (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.

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.

Key word : Rice, Senescing leaf, Proteolysis, GA_3, ABA

Leaf senescence, as most commonly measured by the changes in chlorophylls and proteins, is affected by a variety of internal and external conditions, the most notable of the former being the level and/or activity of plant hormones. Generally, cytokinin acts as a retardant and abscisic acid (ABA) as a promoter of rice leaf senescence[2,8,12,13,15,16,22,23]. The role of gibberellic acid (GA_3) in leaf senescence is less clear[6,8,14,19]. Changes in chlorophylls and proteins have been measured after applying GA_3 and ABA to intact[1,8,22] and detached[13,15,16,23] rice leaves. However, the results differ between different developmental

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stages and environmental conditions. Few workers reported changes in the activity of proteolytic enzymes to account for the measured changes in proteins[2,20].

We have previously reported that NaCl and KCl increase chlorophyll breakdown and proteolysis of senescing rice leaves[10,11]. The mechanisms in volved in the increase in proteolysis include the increased synthesis of proteolytic enzymes and their activity. One of the important regulatory mechanisms which increased proteinase activity was the increased efflux of amino acids from senescing leaves to the incubation media[10]. The increase in amino acid efflux in the presence of the salts is similar to the effect of ABA on senescing leaves ; ABA also increases the leakage and/or exudation of solutes from senescing leaves[5,15,24,25,26,27]. Since the level of ABA increases in senescing leaves of rice[2] and oat[7], it was our interest to understand how hormones, especially ABA, affect the salt-enhanced proteolysis in senescing rice leaves.

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. Results show that, while GA_3 does not affect segment senescence significantly, the effect of ABA is additive to that of KCl in promoting proteolysis. The additive effect was confirmed in leaf protein decline, amino acid efflux, water absorption and Rubisco-degrading endoproteinase (R−endo) activity. The results on amino acid efflux further strengthens our previous conclusion[10,11] *i. e.*, it can be an important mechanism in regulating proteinase activity of senescing rice leaves.

Materials and Methods

Growth of rice seedlings (*Oryza sativa* L. cv. Chilsung) for 16 to 18 days and the preparation of leaf segments from the second true leaves were as described previously[10,11]. Five 5−cm−long segments 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 (100μM), and ABA (1μM) were made up in the control buffer. Leaf segments were light-incubated at 28℃ for 8 days : one-fourth of them were harvested every other day while the rest were supplied with fresh test solutions. When more than 5 leaf segments were needed for enzyme extractions, segments were pooled from separate tubes containing 5 segments each.

Crude extracts made with 5 mM sodium phosphate, pH 7.0, were passed through a PD−10 column (Pharmacia−LKB), and the first 3. 5−ml protein fraction was used as an enzyme source. In addition to hemoglobin, ribulose−1,5−bisphosphate carboxylase/oxygenase (Rubisco) protein, purified from 8− to 12−day−old seedling leaves, was used as a substrate[10]. Reaction was linear for at least 4 hours, but was run for 3 hours at 40℃. The ninhydrin−positive compounds (NPCs) were measured from the trichloroacetic acid (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 12 M HCl hydrolyzates was taken to be a measure of R−endo activity[9,10,11,21]. The NPCs are expressed as the amount equivalent to L−leucine (Leu eq). Proteins and amino acids were measured, using bovine serum albumin and

L-leucine as a standard, respectively[10,11]. Both fresh weights of leaf samples and volumes of incubation media remained in the tubes were measured when leaf segments were harvested every other day. Differences in water loss between the tubes without leaves and those with leaves were considered to indicate the amount of water absorbed (and transpired) by leaf segments in 2 days. Experiments were repeated at least 5 times with 3 replications each.

Some results were statistically analyzed with the Duncan's multiple range test(DMRT) at 5% significance level. Otherwise, standard deviations were 4 to 8% of the mean, and the averages of the means of the separate experiments are presented.

Results

When compared with the controls, the presence of GA_3 appeared to affect leaf proteolysis little (Table 1). Amino acids accumulated less in GA_3-treated leaves at day 2 than in the control leaves. Combined treatment of GA_3 with KCl resulted in lower proteolysis and less accumulation of amino acids than in KCl alone.

ABA increased proteolysis and by day 8, the ABA-treated leaves contained 11% less protein than control leaves (Table 1). The initial increase in proteolysis in the presence of ABA was reflected by the increased accumulation of amino acids in leaf samples of days 2 and 4. When leaf segments were exposed to ABA combined with KCl, an additive effect was observed not only in promoting proteolysis, but in accumulating amino acids.

Leaf amino acids increased until day 6, followed by a decline at day 8 when leaves were treated with ABA alone or with ABA and KCl.

A control leaf segment absorbed 231μl of water in 8 days (Table 2). Addition of KCl reduced water absorption of leaf segments by some 20%. Neither GA_3 in the media affected water absorption significantly, nor did the addition of GA_3 to KCl-treated leaves affect

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
		%[2]			
Protein	Control	85a	61a	38a	29a
	KCl	70c	38c	24bc	16b
	GA_3	88a	62a	38a	28a
	ABA	79ab	55b	35a	18b
	GA_3+KCl	72bc	40c	30ab	19b
	ABA+KCl	68c	35c	25bc	16b
Amino acid	Control	187c	306c	453a	456a
	KCl	253b	325b	427b	388bc
	GA_3	163c	319bc	453a	460a
	ABA	256b	359b	466a	425ab
	GA_3+KCl	244b	284ab	442ab	412b
	ABA+KCl	319a	413a	428b	408b

1) Concentrations used : GA_3, 100μM ; ABA, 1μM ; KCl, 50mM.

2) 100% at day 0 : 239μg proteins and 28μg Leu eq amino acids per leaf segment. Values followed by the same letter within a column of each compound are not significantly different by DMRT at 5% level.

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

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

1) See table 1 for chemical concentrations used.

the KCl−reduced water absorption. ABA reduced water absorption by some 10 to 15%, and the combined treatment of ABA with KCl reduced it by more than 30%.

KCl and ABA increased the efflux of amino acids into the media, whereas GA_3, either with or without KCl, did not affect it (Table 3). On the other hand, the combined treatment of ABA and KCl increased the efflux more than either one alone.

In the presence of GA3, total activity of

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	0.56c	2.20d	3.28c	1.46d
KCl	1.24b	4.84c	7.70b	7.38c
GA_3	0.52c	2.28d	3.09c	2.01d
ABA	1.36b	6.38b	8.94a	10.26b
GA_3+KCl	1.18b	4.77c	7.82b	7.66c
ABA+KCl	1.92a	7.88a	9.05a	11.82a

1) See table 1 for chemical concentrations used.

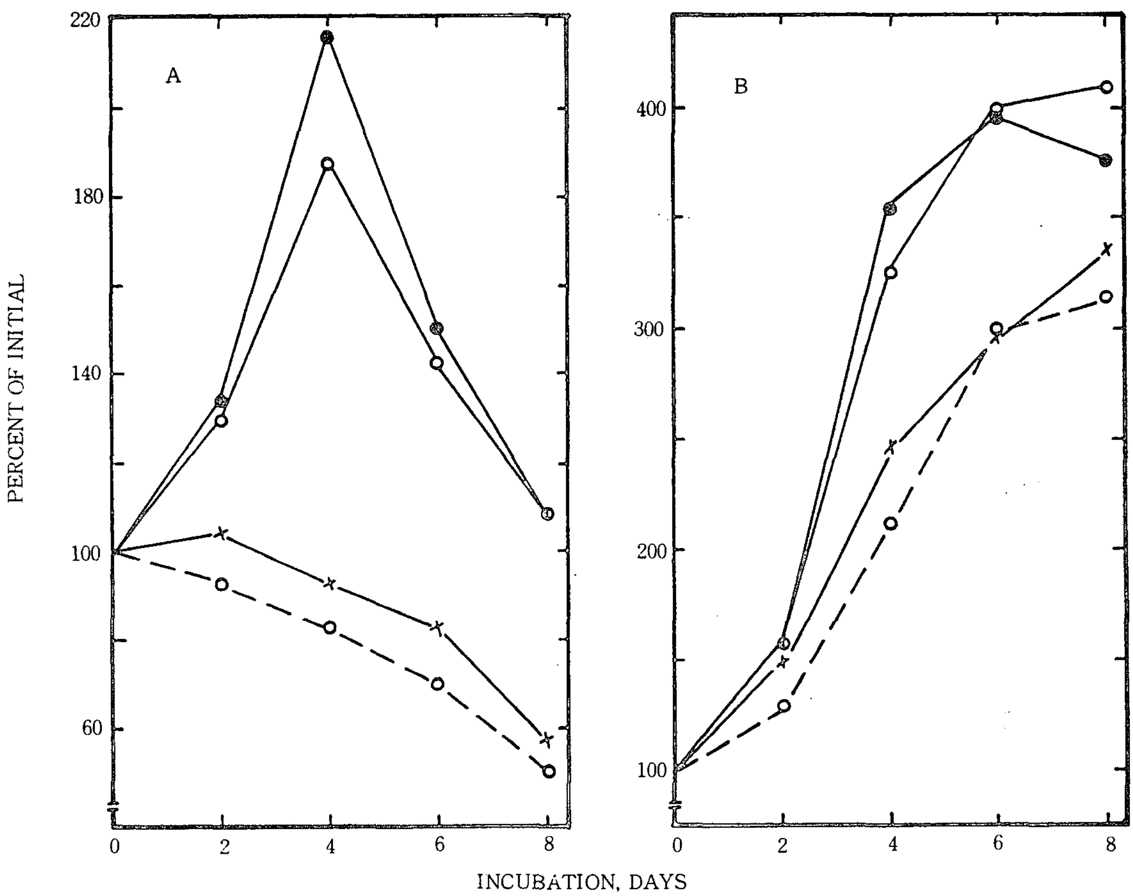

Fig. 1. Relative changes in total(A) and specific(B) activities of H−exo of rice leaves as affected by different incubation media. Leaf segments were light-incubated in the media containing 50mM KCl(… o …), 100μM GA_3(− o −), and a combination of two(−x−). Five mM sodium phosphate buffer at pH7.0 served as controls(− o −). Day 0 values(100%) of total and specific activities were, in μg Leu equ, 12.0 per leaf segment and 50.2 per mg protein, respectively.

H−exo increased almost 2−fold by day 4, followed by a decline to almost the initial level by day 8 (Fig. 1). Although H−exo activity increased in the presence of GA_3, it was lower than in control leaves. KCl lowered total activity gradually to 50% of the initial activity by day 8. Despite that GA_3 alone decreased H−exo activity slightly, addition of GA_3 to KCl−treated leaves did not decrease H−exo activity more than KCl alone. On the contrary, GA_3 slightly counteracted the depressing effect of KCl. Decline in total H−exo activity was evident in the presence of KCl alone or combined with GA_3, specific activity nonetheless increased more than 3−fold by day 8 due to a rapid decline in leaf proteins.

Relative changes in total activity of R−exo (Fig. 2) exhibited a similar pattern to those of H−exo. However, the initial day 0 activity of R−exo was lower, the magnitude of the change throughout the incubation was less, and the activity peak is shown 2 days later than H−exo (Fig. 1).

Addition of GA_3 tended to prevent the

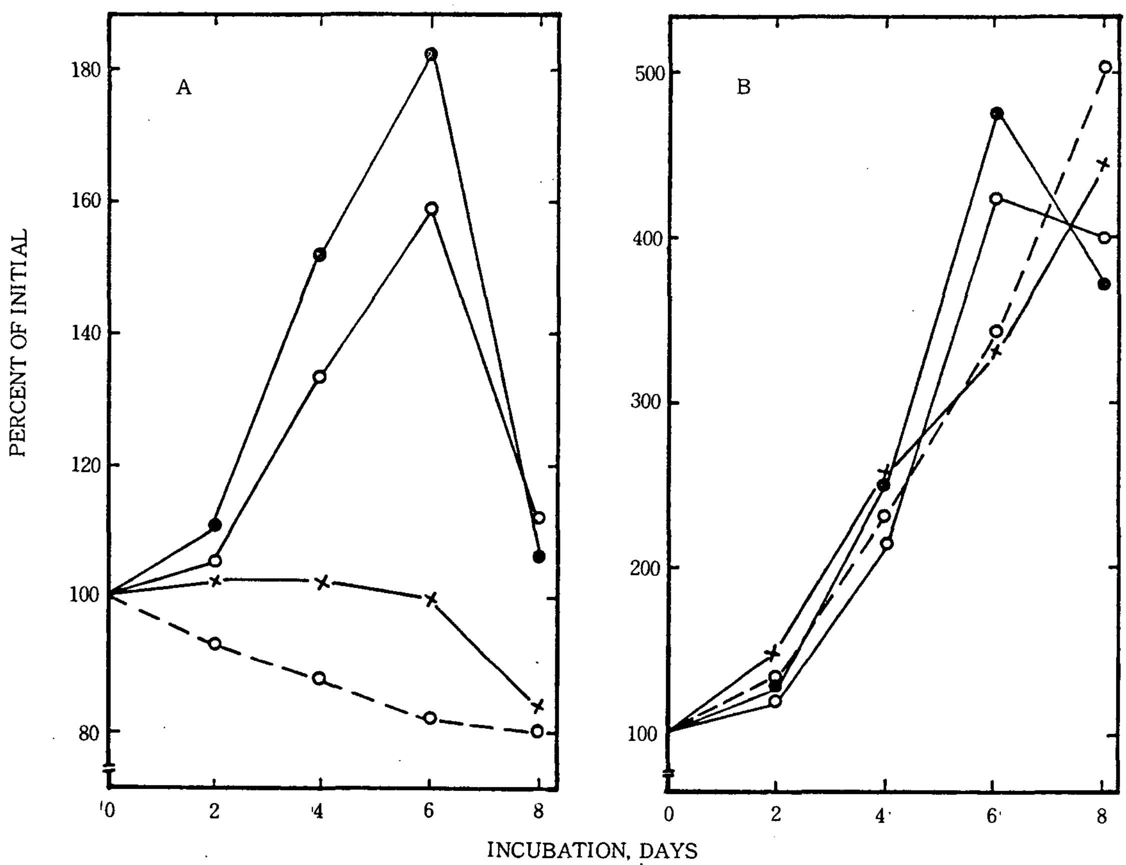

Fig. 2. Relative changes in total(A) and specific(B) activities of R−exo of senescing rice leaves as affected by different incubation media. Leaf segments were light-incubated in the media containing 50mM KCl(⋯ ○ ⋯), 100μM GA_3(− ○ −), and a combination of two(−x−). Five mM phosphate buffer at pH7.0 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.

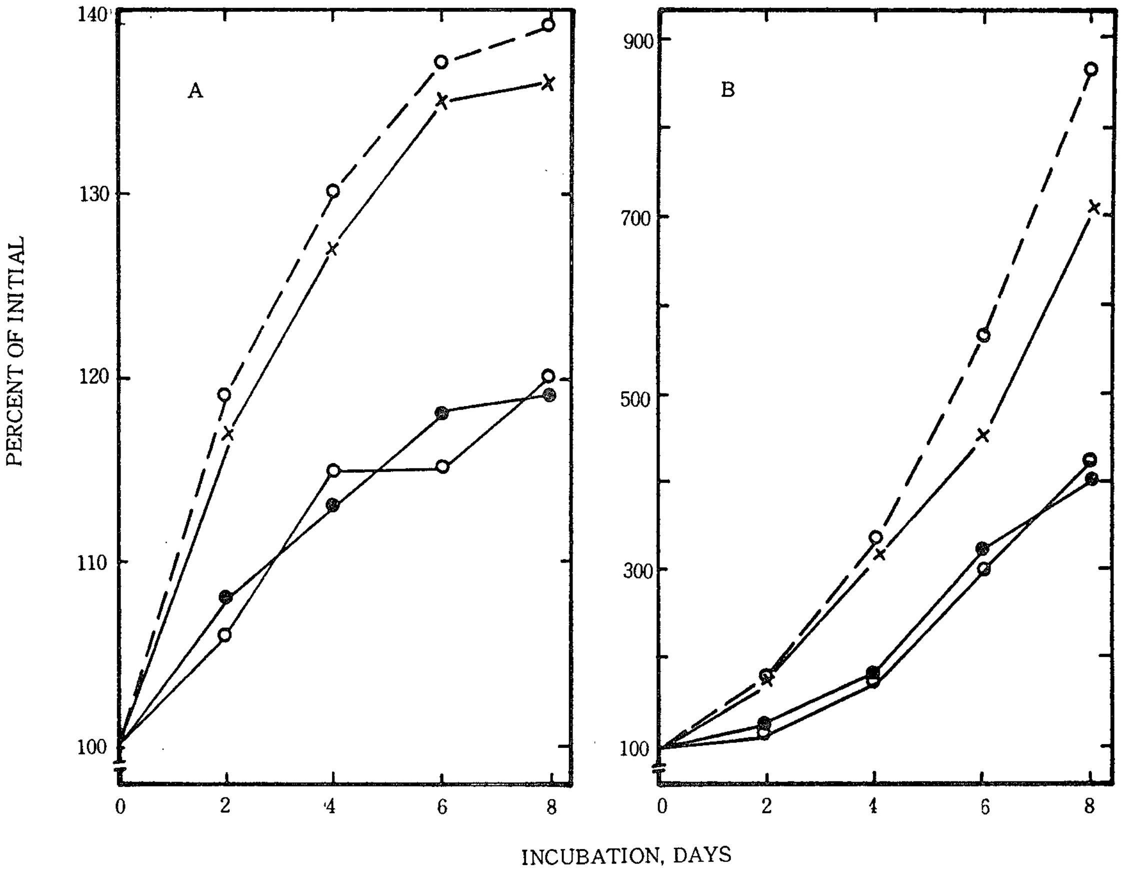

Fig. 3. Relative changes in total(A) and specific(B) activities of R−endo of senescing rice leaves as affected by GA_3 and KCl. Leaf segments, taken from the second true leaves of 16-day-old seedlings, were light-incubated in various media containing 50mM KCl(··· o ···), 100μM GA_3(− o −), and a combination of two(−x−). Five mM sodium phosphate buffer at pH7.0 served as controls(− o −). Purified Rubisco 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.

KCl−induced activity decline from occurring until day 6. KCl increased R−endo activity 39% by day 8 (Fig. 3). GA_3 alone did not affect the activity compared with the controls, the activity being increased by 20% in both GA_3−treated and control leaves. Addition of GA_3 to KCl−treated leaves exhibited about the same level of activity as those treated with KCl alone. Stimulation of R−endo activity by KCl, either with or without GA_3, increased specific activity while GA_3 alone had no effect.

In the presence of ABA, relative changes in total activity of H−exo (data not shown) were similar to those of the R−exo (Table 4). Unlike GA_3, ABA reduced R−exo activity as did KCl. When combined with KCl, ABA counteracted the suppressing effect of KCl on R−exo activity until day 6. ABA alone slightly increased R−endo activity as did KCl. However, the combined treatment of KCl and ABA increased the activity more

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				Specific Act. at day			
		2	4	6	8	2	4	6	8
					%[2)]				
Exo	Control	110	152	182	107	129	249	478	371
	KCl	93	88	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, 1μM ; KCl, 50mM.
2) See Figs. 2 and 3 for 100% activity at day o of R−exo and R−endo, respectively.

than either one alone. This increase in total activity by the combined treatment resulted in more than a 9−fold increase in specific activity.

Discussion

GA_3 has generally been considered a leaf senescence retardant[19,24)], although species-specific responses has been observed[6)].

Little effect of GA_3 was observed in the magnitude of proteolysis (Table 1) and amino acid efflux (Table 3). The activities of H−exo (Fig. 1) and R−exo (Fig. 2) as well as the R−endo (Fig. 3) in the presence of GA_3 never exceeded the levels of control leaves. More than a 50% increase in caseolytic activity by GA_3 was reported in rice[15)], however, we could not find such an increase in our system.

When leaves were treated with both GA_3 and KCl, all the measured changes were determined largely by KCl with little GA_3 effect. An exception to this was the apparent alleviation of KCl−suppressed activities of exoproteinases by GA_3 (Figs. 1 and 2), although we consider that this would not have affected overall proteolysis significantly. Unless there is a concomitant increase in endoproteinase activity, which was not the case (Fig. 3), the exoproteinases alone would not do much to increase overall proteolysis. We therefore concluded that GA_3 affects detached rice leaf senescence little, and this conclusion agrees with that of Harada and Nakayama[8)].

ABA alone increased proteolysis with concomitant increase in leaf amino acids (Table 1) and in amino acid exudation (Table 3). Our results are in agreement with other reports on the promoting effect of ABA on leaf senescence of a variety of plants.[4,5,18,19,24,25,26)]. As was the case in senescing oat leaves[7)], ABA levels increase in senescing flag leaves of field-grown rice plants[2)]. ABA may induce a rapid acceleration of cellular degeneration[15,17)], causing the senescing flag leaves of rice[15)] and detached leaves of oat[26,27)] to leach out the solutes.

Effect of ABA on KCl−increased proteolysis was of great interest. ABA appeared to be additive to KCl in increasing proteolysis especially during early stages of incubation (Table 1). The additive effect of ABA was also found in amino acid efflux (Table 3) and in R−endo activity (Table 4). This effect of ABA contrasted to that of GA_3 in that no additive effect, or negative effect for that matter, was observed between KCl and GA_3 in proteolysis (Table 1), amino acid exudation (Table 3), and in R−endo activity (Fig. 3). As with ABA (Table 4), GA_3 also counteracted the suppressing effect of KCl on R−exo activity (Fig. 2). However, the effect of GA_3 differed from that of ABA in that GA_3 did not affect KCl−enhanced R−endo activity (Fig. 3) while ABA increased it further (Table 4). Such an additional increase in R−endo activity with ABA and KCl, combined with some recovery from KCl−suppressed R−exo activity with ABA, would have affected the overall increase in proteolysis significantly (Table 1).

It appears that there is a close relationship between protein decline and the extent of water absorption of leaf segments. When proteolysis was promoted by KCl in the media, there was a consistent decrease in water absorption (Table 2) and fresh weight of the segments (data not shown).

Also, the additive effect of ABA to that of KCl in promoting proteolysis (Table 1) was associated with a further reduction in water absorption of the segments (Table 2). However, this is not to suggest that proteolysis requires complete desiccation of the leaves. The degree and the rate of desiccation may be quite important. When cysteine was added to the incubation media, there was a concentration-dependent, rapid increase in proteolysis of rice leaf segments (Kang, unpublished results). Although the increased synthesis and/or activity of cysteine-dependent proteinases may be involved here[3,13], the cysteine-increased proteolysis at the early stage of senescence was followed by no such changes in proteolysis at late senescence, due primarily to severe desiccation of leaf segments.

In summary, the KCl−enhanced proteolysis of senescing leaf segments can further be increased by adding ABA to the KCl−containing media. This additional increase in proteolysis can be explained by a further (a) increase in the activity of R−endo with some recovery from the KCl−suppressed exoproteinase activity, (b) increase in the efflux of protein hydrolyzates, and (c) reduction in water absorption of the segments.

Although ABA is known to alter membrane permeability of senescing leaves[5,15,17,26,27], and the increase in amino acid efflux is indicative of this, it is not known how much the alterations in membrane permeability of the cells affected the additive effect of ABA we observed. Also unknown is the mechanism by which ABA affects *de novo* synthesis of proteinases, which is known to be increased by KCl[10,11].

摘　　要

水稻 葉切片의 蛋白質含量 감소는 KCl처리에 의해 촉진된다. 본 연구는 KCl이 增加시키는 단백질 分解作用을 더욱 促進시키기 위한 시도의 하나로 GA_3와 ABA를 KCl과 混合處理한 다음, 이들이 老化葉의 단백질 분해작용을 調節하는 기작을 구명하기 위하여 수행하였다. 파종후 16~18일된 實生의 제2본엽을 5cm 길이로 잘라 test 溶液에 배양하는 8일간 蛋白質, amino酸, 吸水量, 蛋白質 分解酵素의 活性과 단백질 분해산물인 amino산의 流出量 변화 등을 조사하였다.

1. GA_3 단독처리는 잎의 蛋白質 減少에 큰 영향을 미치지 않았고, KCl과 혼합처리되어도 KCl 단독처리와 비교할 때 有意的인 변화를 유기하지

않았다. 그러나 ABA 단독처리는 蛋白質分解, amino산의 流出 및 endoproteinase 活性을 현저히 增加시켰고, ABA와 KCl의 혼합처리는 이들의 增加에 相助的이었다.

2. KCl은 Rubisco를 分解하는 exoproteinases의 活性을 감소시키는데 반해, GA_3나 ABA 添加는 모두 이의 減少를 緩和시키는 효과가 있었다. 그러나 KCl이 처리된 잎의 endoproteinase 활성은 ABA와의 혼합처리에 의해서만 相助的인 증가를 나타내어 이러한 效果가 없는 GA_3와는 그 機能이 對照的이었다.
3. 培養期間중 잎의 吸水量이 낮을수록 蛋白質 減少量이 많고 그 速度도 빨랐다. ABA와 KCl의 혼합처리로 단백질이 가장 많이 감소하였던 잎의 吸水量이 가장 낮아, 蛋白質 分解作用과 吸水量 사이에 깊은 관련이 있음을 示唆하였다.
4. 따라서 KCl에 의한 水稻 葉切片의 蛋白質分解 促進은 endoproteinases 活性과 amino酸의 流出 增加 및 培養初期의 吸水量 減少 등에 그 원인이 있고, ABA는 이러한 변화에 相助的으로 作用하나 GA_3는 큰 영향이 없음을 알 수 있었다.

Literature Cited

1. Biswas, A. K. and M. A. Choudhuri. 1977. Regulation of leaf senescencein rice by hormone sprayed at different developmental stages and it seffect on yield. Indian J. Agric. Sci. 47 : 38－40.
2. Biswas, A. K. and M. A. Choudhuri. 1980. Mechanism of monocarpic senescence in rice. Plant Physiol. 65 : 340－345.
3. Cheng, S. H. and C. H. Kao. 1984. The role of proteolytic enzymes in protein degradation during senescence of rice leaves. Physiol. Plant. 62 : 231－237.
4. Colquhoun, A. J. and J. R. Hillman. 1972. The effects of abscisic acid on senescence in leaf discs of radish, *Raphanus sativus* L. Planta 105 : 213－224.
5. DeLeo, P. and J. A. Sacher. 1970. Control of ribonuclease and acid phosphatase by auxin and abscisic acid during senescence of Rheo leaf sections. Plant Physiol. 46 : 806－811.
6. Fletcher, R. A. and D. J. Osborne. 1965. Regulation of protein and nucleic acid synthesis by gibberellin during leaf senescence. Nature 207 : 1176－1177.
7. Gepstein, S. and K. V. Thimann. 1980. Changes in abscisic acid content of oat leaves during senescence. Proc. Nat'l. Acad. Sci. 77 : 2050－2053.
8. Harada, J. and H. Nakayama. 1982. Effect of gibberellin applications after heading on the longevity of rice leaves with reference to grain development. Bull. Hokuriku Nat'l. Agric. Exp. Stn. 24 : 85－102.
9. Kang, S. M., H. Matsui, and J. S. Titus. 1982. Characteristics and activity changes of proteolytic enzymes in apple leaves during autumnal senescence. Plant Physiol. 70 : 1367－1372.
10. Kang, S. M. and J. S. Titus. 1989. Increased proteolysis of senescing rice leaves in the presence of NaCl and KCl. Plant Physiol. 91 : 1232－1237.
11. Kang, S. M. and J. S. Titus. 1992. Effect of phosphate on proteolysis of senescing rice leaves. Phytochemistry 31 : 401－404.
12. Kao, C. H. 1980. Senescence of rice leaves. IV. Influence of benzyladenine on chlorophyll degradation. Plant Cell Physiol. 21 : 1255－1262.
13. Kar, M. and D. Mishra. 1977. Protease activity during rice leaf senescence. Biol. Plant. 19 : 365－369.
14. Kwon, Y. W. and S. M. Kang. 1992. Senescence of rice leaves and its regulation by inhibitors of GA biosynthesis during

vegetative growth and grain filling. The 1st ACSC Symp. Abstr., pp. 94－95.
15. Maitra, N. and S. P. Sen. 1987. Hormonal regulation of source-sink relationship : Effect of hormones on excised source and sink organs of cereals. Plant Cell Physiol. 28 : 1005－1012.
16. Mishra, D. and P. Pradhan. 1973. Regulation of senescence in detached rice leaves by light and kinetin. Exp. Geront. 8 : 153－155.
17. Mittelheuser, C. J. and R. F. M. Van Steveninck. 1971. The ultrastructure of wheat leaves. I. Changes due to natural senescence and the effects of kinetin and abscisic acid on detached leaves incubated in the dark. Protoplasma 73 : 239－252.
18. Mohan Rao, K. and P. A. Khan. 1983. Interaction of ABA and light on leaf senescence in rice. Plant Cell Physiol. 24 : 577－580.
19. Nooden, L. D. 1988. Abscisic acid, auxin, and other regulators of senescence. In : Nooden, L. D. and A. C. Leopold (Editors), Senescence and Ageing in Plants. Academic Press, San Diego, pp. 329－368.
20. Perez, C. M., G. B. Cagampang, B. V. Esmama, R. U. Monserrate, and B. O. Juliano. 1973. Protein metabolism in leaves and developing grains of rices differing in grain protein content. Plant Physiol. 51 : 537－542.
21. Ragster, L. E. and M. J. Chrispeels. 1981. Hemoglobin-digesting acid proteinases in soybean leaves. Plant Physiol. 67 : 110－114.
22. Ray, S., W. A. Mondal, and M. A. Choudhuri. 1983. Regulation of leaf senescence, grain-filling and yield of rice by kinetin and abscisic acid. Physiol. Plant. 59 : 343－346.
23. Singh, N. and D. Mishra. 1975. The effect of benzimidazole and red light on the senescence of detached leaves of Oryza sativa cv. Ratna. Physiol. Plant. 34 : 67－74.
24. Tan, Z－Y., B. Veierskov, J. Park, and K. V. Thimann. 1988. Multiple actions of abscisic acid in senescence of oat leaves. J. Plant Growth Regul. 7 : 213－226.
25. Thimann, K. V. 1980. The senescence of leaves. In : Thimann, K. V. (Editor), Senescence in Plants. CRC Press, Boca Raton, pp. 85－116.
26. Trippi, V. and K. V. Thimann. 1983. The exudation of solutes during senescence of oat leaves. Physiol. Plant. 58 : 21－28.
27. Veierskov, B. and K. V. Thimann. 1988. The control of protein breakdown and synthesis in the senscence of oat leaves. Physiol. Plant. 72 : 257－264.