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Ribonucleic Acid and Ribonuclease Activity in the Developing Shoot of Rice Plants at Low Temperature

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벼의 幼芽期에 冷害가 RNA 및 RNase 活性度에 미치는 影響

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Summary

The qualitative and quantitative changes in RNA in terms of RNase activity of rice plants subjected to the chilling temperature were studied.

The total RNA level increased at the early stage and thereafter decreased continuously while the progress of the chilling injury.

The change of total RNA was mainly dependent upon the change of ribosomal RNA with soluble RNA less changed.

Parallelism between total RNA level and RNase activity was observed at the early stage of chilling injury, while the inverse relationship of RNA-RNase was seen in the later stage.

Our observations indicate that synthetic function of RNase may be more closely related to ribosomal RNA than soluble RNA.

Introduction

Many plant species are liable to injury by low but non-freezing temperature(chilling injury.) Low temperature produces a number of metabolic changes within plant tissues before any visual symptoms occur (1, 2, 10).

Many investigators have reported that chilling and frost injuries induce a substantial increase in protein and RNA in woody plants (11, 12, 13) and alfalfa (14), and decrease in RNase activity in mimosa seedlings (15). In cotton plants, however, there occurs decrease in RNA during the chilling (1).

The correlations between RNA level and RNase activity have been noted by many workers (3, 4, 6). Under the specific growth conditions, there existed a linear relationship between RNA level and RNase activity, while under other conditions RNA-RNase were inversely related. These relationships were associated by several workers with the role of RNase in synthesis or degradation of RNA (5-8). At the same time, some of them suggested that the ratio of soluble RNA (s-RNA) to ribosomal RNA (r-RNA) and of soluble RNase to particulate or bound RNase might regulate the RNA-RNase relationship (8, 9). Of considerable interest therefore would be determinations of s-

and r-RNA under specific physiological growth conditions.

The present report is concerned with the changes in total RNA, specific RNA components and RNase activity in the early developmental stage of rice plants subjected to chilling temperature.

Materials and Methods

Plant Materials

Rice grains (*Oryzae sativa* L. var. Jin Heung and Su Won 215) supplied by Agronomy Department, College of Agriculture, Seoul National University were used.

The grains were sterilized with 0.5% sodium hypochlorite, soaked at 28°C for 24 hours and germinated on the damp sponge in the dark at 28°C for 60 hours. Following the germination period, growth was continued in the dark at 28°C and 13°C respectively without any supplement of the external nutrients. Plants were harvested at daily intervals. Shoots were removed from the other parts and were then either immediately subjected to analysis or stored at -20°C, if necessary.

Estimation of RNA

The RNA estimation was performed by a modified Schmidt-Thannhauser procedure (8). The shoots were dropped for 2 min into boiling 95% ethanol and homogenized with 70% ethanol at 0°C.

The acid soluble materials were extracted with ice-cold 5%-trichloroacetic acid. The extraction of the residue was continued successively with 95% ethanol and ethanol-ether (2 : 1). The residue was then incubated with 0.3 N-KOH at 37°C for 16 hours. The resultant was acidified to pH 2 with 0.5N perchloric acid.

The amount of RNA in the supernatant was determined at the wavelength of 260 mμ with Beckman DU spectrophotometer.

Determination of RNase Activity

RNase activity was assayed at pH 5.4 after the methods of Dalby and Davies (16). The enzyme extract was prepared in 0.05M tris buffer (pH 7.5) containing 0.5 MKCl at O°C. One m*l* of the above enzyme extract, 1 m*l* of 0.1M sodium citrate buffer (pH 5.4) and 0.5 m*l* of 0.4% purified yeast RNA substrate were mixed for the assay of RNase activity and incubated at 37°C for 25 min. Then 0.5m*l* of 0.75% uranyl acetate in 25% perchloric acid were added and centrifuged. The digested substrate was diluted 15 times with distilled water and determined at 260mμ against zero time incubation.

Extraction of Nucleic Acids for MAK* Chromatography

Nucleic acids were prepared by the sodium dodecyl sulfate (SDS)-phenol method (17). The frozen tissue was homogenized in a medium containing 2 vol. of water-saturated phenol and 1 vol. of the versene buffer (pH 5.0: 0.01 M versene, 0.1 M sodium acetate and 0.1 Msodium chloride). SDS and bentonite were added to the versene buffer in the concentrations of 1% and 0.5% respectively. The homogenate was centrifuged at 10,000 G for 20 min. The aqueous phase was removed and the under layer (phenol phase) was treated with a half vol. of versene buffer containing SDS and bentonite. The combined aqueous phase was extracted twice with 3 vol. of anhydrous ether to remove the residual phenol. Nucleic acids were precipitated from aqueous phase with 2 vol. of 95% ethanol.

The precipitates were purified twice by dissolving in the versene buffer and subsequentely precipitating with cold ethanol and used for application on MAK column.

MAK Chromatography

Nucleic acids were fractionated on MAK column according to the method of Mandell and Hershey (18). Bovine serum albumin Fraction V was estrified with absolute methanol. The methylated album in powder was used at the concentraction of 1% aqueous solution for a column material.

The MAK column was composed of 3 layers and packed under pressure.

The bottom layer contained approximately 2.5 mg of methylated albumin per gram of kieselguhr and the middle layer similarily 0.7 mg/g.

* Methylated Albumin Kieselguhr

The kieselguhr in the top layer acts as a protective layer. The nucleic acid dissolved in 0.05M phosphate buffer (pH 6.7) containing 0.1M saline was applied on MAK column.

Elution was carried out with a sodium chloride linear gradient concentration from 0.1 M to 1.2 M in 0.05 M phosphate buffer (pH 6.7) under pressure to give a flow rate of 25m*l* per hour at 25°C. Fractions were 4m*l* per tube. Nucleic acid of the each fraction was estimated by absorbance at 260mμ.

Results.

Changes in Total RNA Level;

Daily changes in total RNA level of both the chilling resistant variety, Jin Heung, and the chilling sensitive, Su Won 215, are shown in Table 1.

Table 1. Changes in total RNA content in rice plants at different growth temperature.

Time		RNA (A_{260}/g. fr. wt.)					
days after sowing	days after treatment	Jin Heung			Su Won 215		
		control	treated	%*	control	treated	%
3		0.75			0.90		
4		0.80			1.02		
5	1	0.91	0.95	4.4	1.22	1.20	−16.4
6	2	1.10	1.42	29.1	1.29	1.30	1.0
7	3	1.25	1.51	20.8	1.31	1.55	18.3
8	4	1.55	1.60	3.2	1.50	1.56	4.0
9	5	1.42	1.30	−8.4	1.62	1.36	−13.6
10	6	1.41	1.20	−14.8	1.42	1.15	−18.9
11	7	1.20	1.01	−15.9	1.28	1.01	−21.1

control; 28°C, treated; 13°C, fr. wt.; fresh weight,

* % increase over control $= \frac{\text{treated-control}}{\text{control}} \times 100$

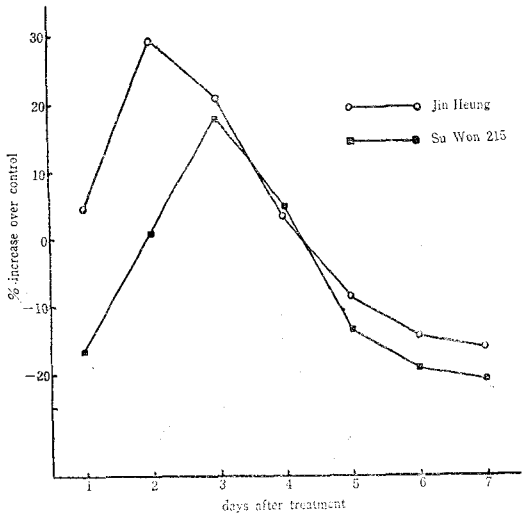

Fig. 1. Changes in the per cent increase in total RNA of the treated over the control.

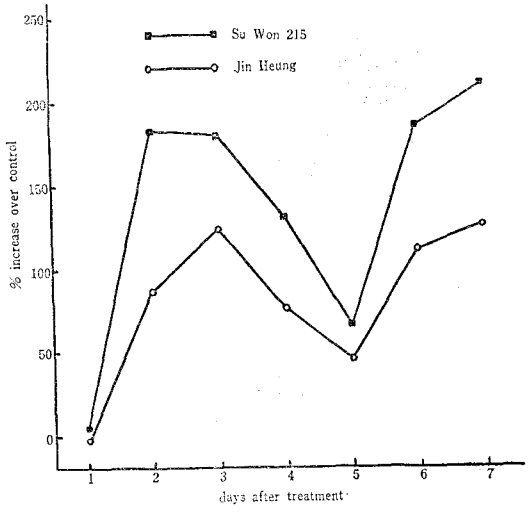

Fig. 2. Changes in the per cent increase in RNase activity of the treated over the control.

In the initial stage of chilling injury, the total RNA level increased more rapidly in the plants subjected to chilling temperature (treated) than in the plants grown at the optimum temperature (control). In the following stage, the total RNA level of the treated began to decrease more rapidly than that of the control. The per cent increase in total RNA level of the treated against that of the control is plotted in Fig. 1. It reached the maximum, 30%, in 2 days after the treatment and gradually decreased to 15% below the control in 7 days after the treatment. A degree of increase in total RNA was more or less higher in Jin Heung than in Su Won 215.

Changes in Total RNase Activity;

The RNase activity of both the treated and the control increased apparently in 3 days after the treatment and then decreased rapidly in 5 d ays after the treatment, as shown in Table 2.

On the 7 th day following the treatment, the

Table 2. Changes in RNase activity in the rice plants at different growth temperature.

Time		RNase activity (A_{260}/g.fr. wt.)					
days after sowing	days after treatment	Jin Heung			Su Won 215		
		control	treated	%	control	treated	%
3		0.30			0.41		
4		0.25			0.29		
5	1	0.82	0.80	−3.0	0.68	0.70	3.0
6	2	1.20	2.24	86.6	1.18	3.34	183.0
7	3	1.08	2.41	123.1	1.36	3.82	180.9
8	4	0.76	1.33	75.0	0.97	2.23	130.0
9	5	0.62	0.90	45.1	0.85	1.40	64.7
10	6	0.45	0.95	111.1	0.60	1.71	185.0
11	7	0.45	1.05	133.3	0.55	1.73	214.6

control; 28C, treated; 13C, % has been defined in Table 1.

RNase activity of the treated re-increased, while that of the control leveled off. The per cent increase in RNase activity of the treated against that of the control is shown in Fig. 2.

The curve shows that the per cent increase in RNase activity of the treated reached 120% in 3 days after the treatment, fell down to 45% in 5 days and thereafter rose up again.

The change in RNase activity of Su Won 215 was more marked than that of Jin Heung.

Fractionation of Nucleic Acids on MAK Column;

s-RNA 1,2 (4S transfer RNA, 5S r-RNA), DNA, r-RNA 1,2,3 (18S r-RNA, 25S r-RNA) of the tissue under study were successfuly fractionated on the MAK column as shown in Fig. 3. The UV (260 mμ) absorbing materials in the early stage of elution are assumed to be degradation products of high molecular weight RNA (21).

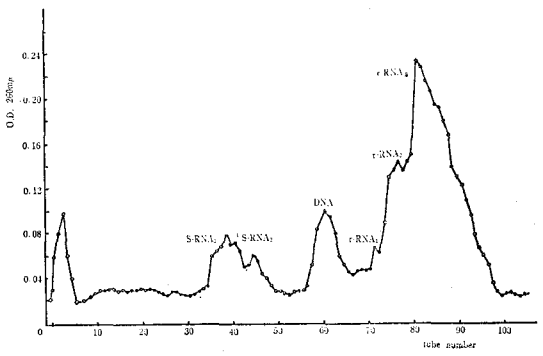

Fig. 3. Profile of MAK column chromatography of rice plant nucleic acids prepared by SDS-phenol method.

The peak corresponding to DNA was identified with the diphenylamine reaction. The relative amount of each fraction was determined by integrating the area under the peaks after MAK chromatography (Table 3). From the total RNA content were computed all of the RNA components in proportion to the relative amount of each RNA fractions. These data are shown in Table 4.

Table 3. Changes in the relative amount of nucleic acid fractions to the total area after MAK chromatography.

days after treatment		total area	Nucleic acid fraction s-RNA	DNA	r-RNA
1	control	100	19.3	15.2	65.5
	treated	100	19.4	14.1	66.5
3	control	100	15.5	11.4	73.1
	treated	100	16.6	12.7	70.7
5	control	100	15.4	11.5	73.1
	treated	100	18.2	15.8	66.0

control; 28°C, treated; 13°C.

Table 4. Changes in RNA fractions in the rice plants at different growth temperature.

days after treatment		total RNA	RNA fraction s-RNA	r-RNA
1	control	0.91	0.204	0.704
	treated	0.95	0.215	0.735
	%	4.6	1.2	3.5
3	control	1.25	0.218	1.033
	treated	1.51	0.287	1.223
	%	20.8	5.4	15.4
5	control	1.42	0.246	1.174
	treated	1.30	0.282	1.017
	%	−8.5%	2.5	−11.0

control; 28 C, treated; 13 C.
% increase over control has been defined in Table I.

An apparently greater change of r-RNA than that of s-RNA is shown in Fig. 4. The amount of r-RNA increased 15% at maximum in 3 days after the treatment and decreased 11% below the control in 5 days after the treatment, whereas s-RNA slightly increased 2-5% above the control throughout the period.

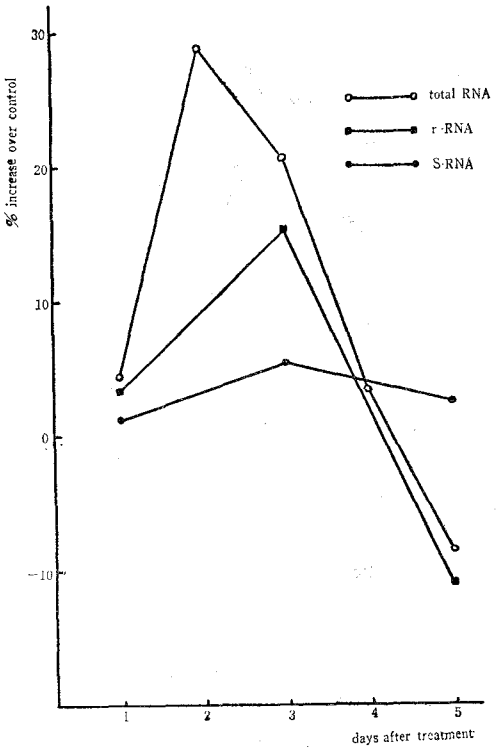

Fig. 4. Changes in the per cent increase of RNA fraction of the treated over the control.

Discussion

In the present experiments, the total RNA content of the cold treated rice plant rose above the level of the control during first 4 days after the treatment and continuously decreased thereafter. This observation is in good agreement with the reports in woody plants and alfalfa (11, 12, 13, 14). It appears that there are flush of metabolic activity at the initial stage of chilling injury, but that with the progress of chilling injury the metabolic activity slows down.

According to the authors' results, the change of total RNA content was mainly due to that of r-RNA content. The s-RNA content changed only slightly throughout the period, regardless of total RNA change. It was reported that r-RNA is the

most responsive species of nucleic acids to the environmental changes (9, 13) and r-RNA is more rapidly degraded when growth has slowed down (19).

RNase activity was obviously higher in the injured plant than in the control in the present study. The result is contrary to the report that there were decreases in RNase activity with increasing cold resistance in mimosa seedlings (15). But, in the fungi (*Basidiomycete*) cultured at the low temperature (0°C), the increase in RNase activity was parallel to the increase in total RNA content at the early stage(20).

In relation between total RNA level and RNase activity, the RNA-RNase parallelism was observed in the early stage of chilling, whereas this relationship did not hold in the later stage. It seems to imply that RNase would exhibit synthetic as well as hydrolytic function on RNA during chilling injury. Some investigators (9, 22) suggested that the syntheses of s-RNA and r-RNA were independently regulated by the enzymes involved in the synthesis of RNA fractions.

Our observations naturally lead to the possibility that synthetic function of RNase is more closely related to r-RNA than s-RNA.

During the cold injury, both varieties, a cold resistant and a cold sensitive ones, showed similar tendency at an early stage. However, the cold resistant Jin Heung showed a higher degree of RNA increase than the cold sensitive Su Won 215. On the other hand, the former indicated lesser increase in RNase activity than the latter.

요 약

벼가 유아기에 냉해(15°C)로 인하여 생장이 정지될 때 RNA와 RNase활성도를 비교 연구하여 아래와 같은 결과를 얻었다.

1. 냉해 초기에는 RNA 함량과 RNase 활성도가 동시에 증가하고 3일 후에는 감소하였으나 5일 후에는 RNA 함량은 계속 감소하는 반면 RNase 활성도는 다시 증가 하였다.

2. 이러한 RNA의 변화는 ribosomal RNA의 변화에 기인함을 확인하였다.

3. RNase는 RNA의 합성 기능도 가지고 있는 것으로 사료되며 이 기능은 soluble RNA보다 ribosomal RNA와 관계가 있는 것으로 추측된다.

References

1. Guinn, G., Crop Sci., **11**, 262(1971)
2. McCown, B.H., T.C. Hall and G.E. Beck, Plant Physiol., **44**, 210(1969)
3. Brody, S. and M.E. Balis, Nature, **182**, 940 (1958)
4. Dedoux, L., P. Galand and R. Huart, Biochem. Biophys. Acta, **55**, 97(1962)
5. Markharm, R. and J.L. Strominger, Biochem. J., **64**, 46P(1956)
6. Barker, G.R. and T. Douglas, Nature, **188**, 943(1960)
7. Ingle, J. and R.H. Hageman, Plant Physiol., **40**, 48(1965)
8. Kessler, B. and N. Ingelberg, Biochem. Biophys. Acta, **55**, 62(1962)
9. Kjeldgaard, N.O. and C.G. Kurland, J. Mol. Biol., **6**, 341(1963)
10. Stewart, J.M. and G. Guinn, Plant Physiol., **44**, 605(1969)
11. Li, P.H. and C.J. Weiser, Plant Cell Physiol., **10**, 21(1969)
12. Ibid., Proc. Amer. Soc. Hort. Sci., **91**, 716 (1967)
13. Gusta, L.V. and C.J. Weiser, Plant Physiol., **49**, 91(1972)
14. Jung, G.A. and S.C. Shih and D.C. Shelton, Crybiology, **4**, 11(1967)
15. Ho, T. and G.N. Grown, ibid, **6**, 265(1969)
16. Dalby, A. and I.I. Davies, Science, **155**, 1573 (1967)
17. Click, R. E. and D.P. Hackett, Biochem. Biophys. Acta, **129**, 74(1966)
18. Mandell, J.D. and A.D. Hershey, Anal. Biochem., **1**, 66(1960)
19. Schweizer E. and H.O. Halvorson, Exptl. Cell Res., **56**, 239(1969)
20. Sekhon, A.S. and N. Colotelo, Can. J. Bot., **48**, 1827(1970)
21. Monier, R., S. Naono, D. Hayes and F. Gros, J. Mol. Biol., **5**, 311(1962)
22. Kurland, C.G. and O. Maale, J. Mol. Biol., **4**, 193(1962)