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# The Effect of Indole Acetic and Abscisic Acid on Ribonucleic Acid and Ribonuclease

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## Indole acetic acid와 Abscisic acid가 核酸과 RNase에 미치는 영향에 關하여

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### Summary

Wheat coleoptile sections were treated with either $1.5\times10^{-5}$ M ABA or $5\times10^{-5}$ M IAA in vitro, the results may be summarized as follows,

1. The treatmert of IAA decreased the level of high molecular weight RNA F2 and F3 but that with ABA increased the F4 level.
2. IAA caused an increased activity of G2 isozyme, while ABA suppressed the activity of G3 isozyme.
3. The results may suggest that there may exist common effects of IAA and ABA on RNA and RNase.
4. The latent RNase activity caused by SH blocking reagent (p-hydroxymercury benzoate, Pb et al) was not observed.

### Introduction

The roles played by indole acetic acid (IAA) and abscisic acid (ABA) in RNA metabolism are of current interest and several reviews have been reported elsewhere (1, 2, 3, 4). While Chrispeels and Varner (5) observed that ABA inhibited RNase activity, it has been generally thought that ABA enhances RNase activity and lowers RNA level in plant tissues (1, 2, 3). Many workers have been concerned in the selective effect of ABA on RNA components but the views were somewhat different. According to Khan and Heit (6) in germinating pear embryos, Walton et al (7) in excised embryonic bean axes and Shih et al (8) in the excised buds of potato ABA affects s-RNA, DNA and r-RNA. In pear embryo, however, Khan and Anojulu (9) found that ABA greatly altered the base composition of rapidly labeled RNA species. Leshem and Schwarz (10) saw a substantial decrease in r-RNA compared to other RNA components.

IAA suppresses RNase activity and promotes RNA synthesis as a whole, and a somewhat selective effect of IAA has been noted (11, 12). Key (2) concluded in his review that auxin may generally cause a greater accumulation of r-RNA relative to s-RNA.

However, little attention has been paid to the

effect of the growth regulators on RNase isozymes and high molecular weight RNAs. In the present study the changes in the isozyme patterns and high molecular weight RNAs as well as other RNA components, after IAA and ABA were applied to the wheat coleoptile sections, were investigated.

## Material and Methods

**Plant material;** Seeds of wheat variety, Yuk-Sung #3 were sterilized in the 0.5% sodium hypochlorite solution for 15 minutes and soaked in the tap water for 6 hours. Then the seeds were placed on the damp sponge in a plastic tray, and left in a darkroom at 26—27°C. The coleoptiles were harvested after 72 hours. The coleoptile sections, 15mm pieces cut 3mm below the apex, were treated with either IAA or ABA. The concentration of ABA was $1.5\times10^{-5}$ M and that of IAA $5\times10^{-5}$ M.

**Isolation of RNA for fractionation;** Total nucleic acid was prepared from the coleoptiles by means of sodium dodecyl sulfate (SDS)-phenol extraction. The coleoptiles were homogenized in the presence of acetate buffer (0.1 M, 0.1 M NaCl, 0.01 M EDTA and pH 5.0), phenol, SDS and bentonite (13).

**Estimation of RNase activity;** The coleoptiles were homogenized in 0.1 M acetate buffer (pH 5.0) containing 0.4 M sucrose, 5 ml magnesium acetate, 20 mM KCl, 5 mM mercaptoethanol and 0.2% Triton X-100. The homogenate was centrifuged at 20,000 g for 10 min and the supernatant was used as the crude enzyme solution. For the assay of RNase activity the reaction mixture contained 0.2 ml 0.4% RNA solution, 0.2 ml of the resulting crude enzyme solution and 0.1 ml buffer solution. After incubating the mixture at 37°C for 45 min 1 ml portion of 0.75% uranyl acetate in 25% trichloroacetic acid was added. The mixtures were centrifuged and the supernatant was diluted 15 times with distilled water. The activity was expressed as $A_{260}$/mg of protein/45 min. Protein was estimated after the method of Lowry et al (14).

**Gel electrophoresis of RNA;** A slight modification based on Ikoi's method(15) was employed for the gel electrophoresis of RNA. The RNA preparation was dissolved in the electrophoresis buffer (36 mM Tris, 30 mM sodium dihydrogen phosphate and 1 mM EDTA) supplemented with 0.2% SDS and 5% sucrose. The gels prepared contained 2.5% acrylamide (recrystallized from chloroform) and 0.125% methylene-bis-acrylamide (recrystallized from acetone). Trimethylethyldiamine (TMED) and 10% ammonium persulfate were added at the ratio of 0.033 ml per 1 g of acrylamide. Electrophoresis was continued for 1.5 hours at the room temperature. The gels then were removed from the tubes and stained in 0.2% acridine orange for 2 hours and destained in 10% acetic acid.

**Fractionation of RNase isozymes;** The gels were 6.6% buffered at 71 mM Tris-HCl. Barbiturate (30 mM, pH 7.5) was used as the electrode buffer. Twenty five $\mu$l of the crude enzyme solution were layered on the gel and electrophoresis was carried out, in a refrigerator, at 4 mA per tube for an hour and half. The temperature was maintained at 2°C. The gels were removed from tubes and dipped into the preincubation buffer (0.1 M acetate buffer pH 5.8 containing 0.1 M KCl) at 0°C for 20 min. After washing with the chilled water the gels were incubated for 20 min in a solution containing 4 mg/ml RNA and 50 mM KCl. Finally the gels were dipped into the 0.2% toluidine blue solution in 0.5% acetic acid adjusted to pH 3. After dying for 30 seconds the gels were rinsed in a tap water and stored in 5% perchloric acid.

**Fractionation of RNA on methylated albumin on kieselgur (MAK) column;** The fractionation was done following the method of Mandell and Hershey (16). The salt concentration was in the range of 0.1—1.6 M. and the rate of elution 4.5 ml per 10 min. The absorbance of the eluates was measured at 260 m$\mu$ with Beckman DU spectrophotometer.

**Estimation of RNA content;** It was done with a modified method of Schmidt and Thannhauser (17). One gram of coleoptiles was

homogenized in 0.2 N perchloric acid and centrifuged. The residue was extracted with lipid solvents; once with 95% ethanol, twice with ethanol-chloroform mixture (3 : 1), once with ethanol-ether mixture (3 : 1) and ether. After the alkaline digestion in 0.3 N KOH for 17 hours at 37°C the mixture was acidified with 60% perchloric acid to pH 2.5. The supernatant after centrifugation contained RNA fraction. Spectrophotometric reading was made at 260mμ.

**Densitrometry of electropherogram:** The detailed method has been previously described (18). The enlarged films were scanned at 500mμ with Beckman Analytrol.

**Addition of p-hydroxymercury benzoate;** Toconfirm the presence of the latent RNase, p-hydroxymercury benzoate (0.2ml) was added to the assay mixture (19).

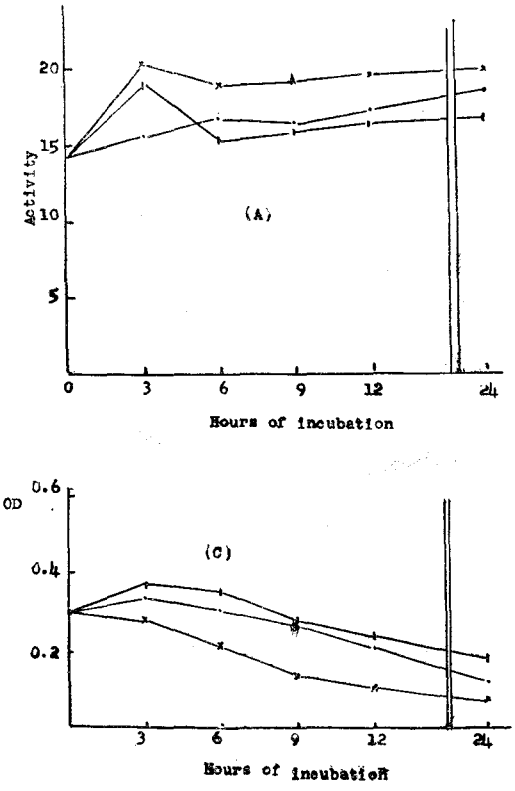
## Result and Discussion

**Effect on total RNase and RNA;** As shown in Fig. 1 the total RNA content of the control and IAA-treated coleptiles increased slightly during the first 3 hours and thereafter slowly decreased, while the ABA-treated exhibited a continuous decrease, markedly after 3 hours of ABA treatment. Both in IAA- and in ABA-treatment the maximum activity of total RNase was observed in 3 hours in contrast to 6 hours in the control. The addition of p-hydroxymercury benzoate lowered the RNase activity.

**The effect on RNase isozymes;** As shown in Fig. 2 and Table 1 the isozyme patterns in both treated samples were not so apparent as in the control. Both IAA and ABA suppressed G1 and G3 isozymes, while they enhanced G2 and G4. The degree of activation or inhibition was varied among isozyme.

**Table 1.** The ratio of each isozyme activity to the total activity measured by integrator.

| | ratio of isozymes(%) | | | |
|---|---|---|---|---|
| | G1 | G2 | G3 | G4 |
| Control | 6.6 | 34.6 | 36 | 23 |
| IAA | 0 | 43 | 28 | 28 |
| ABA | 1 | 48.5 | 24 | 25.7 |



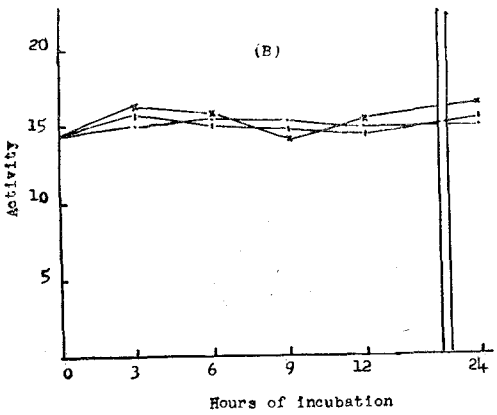




**Fig. 1.** Changes in total RNase activity and total RNA level of wheat coleoptiles along incubation

x——x, $1.5\times10^{-5}$ M ABA; •——• , water; 1——1, $5\times10^{-5}$ M IAA

(A), Total RNase activity. $A_{260}$/mg of protein/45 min. (B,) Total RNase activity after the addition of p-hydroxymercury benzoate (C), Total RNA level

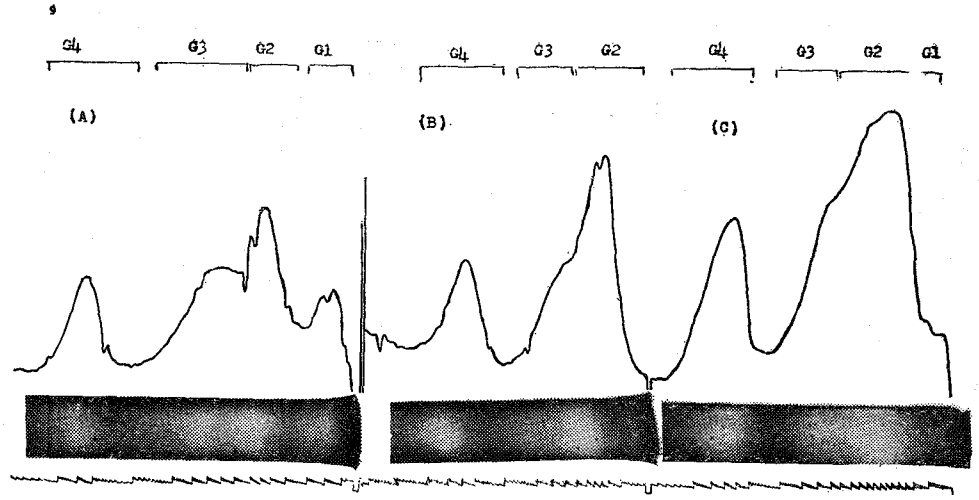



**Fig. 2.** The electropherogram of RNase isozymes extracted from 8-hour-treated wheat coleoptiles. (A), water; (B), $5\times10^{-5}$ M IAA; (C), $1.5\times10^{-5}$ M ABA

**Table 2.** The ratio of each RNA component to total RNA.

| | ratio of each RNA component(%) | | |
|---|---|---|---|
| | *s*-RNA | DNA-RNA | r-RNA |
| Control | 11.5 | 9.5 | 79 |
| IAA | 9.0 | 9.0 | 82 |
| ABA | 20.0 | 9.1 | 63 |

***Effect on* MAK profiles of RNA components;** In IAA treatment(Fig. 3 and Table 2) the r-RNA fraction increased while s-RNA fraction decreased in ABA treatment. r-RNA decreased markedly and s-RNA showed a sharp increase.

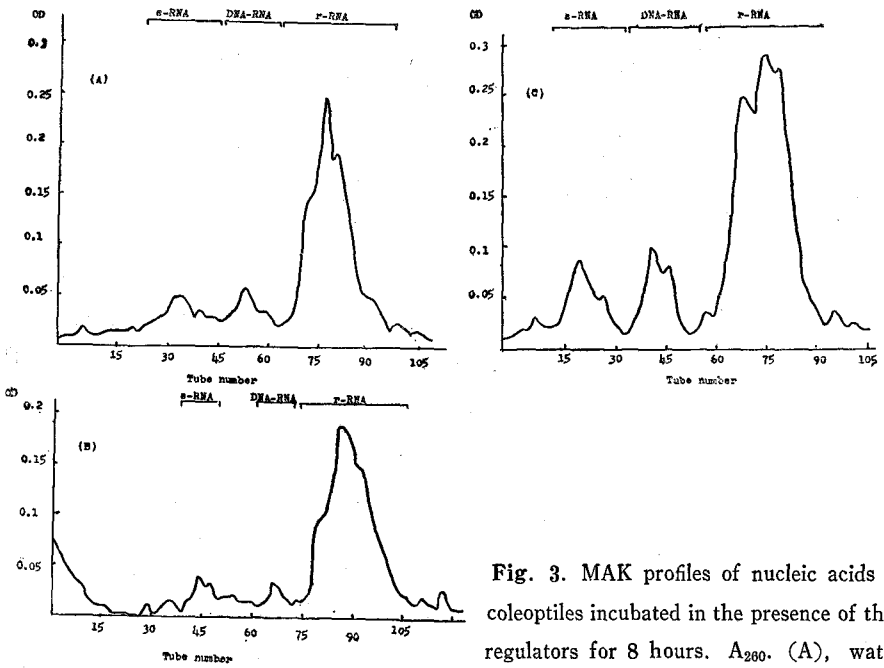



**Fig. 3.** MAK profiles of nucleic acids of wheat coleoptiles incubated in the presence of the growth regulators for 8 hours. $A_{260}$. (A), water; (B), $1.5\times10^{-5}$ M ABA (C), $5\times10^{-5}$ M IAA

**Effect on high molecular weight RNA fractions;** As seen in Fig. 4 and Table 3, Fraction 1 (F1) increased in IAA treatment but remained almost unchanged in ABA treatment. IAA- and ABA-treatment decreased F2 (25S+23S). ABA didn't affect F3 (18S+16S) but IAA reduced it. Both in IAA- and ABA-treatment F4 (13S) increased. The increment was more pronounced in IAA treatment.

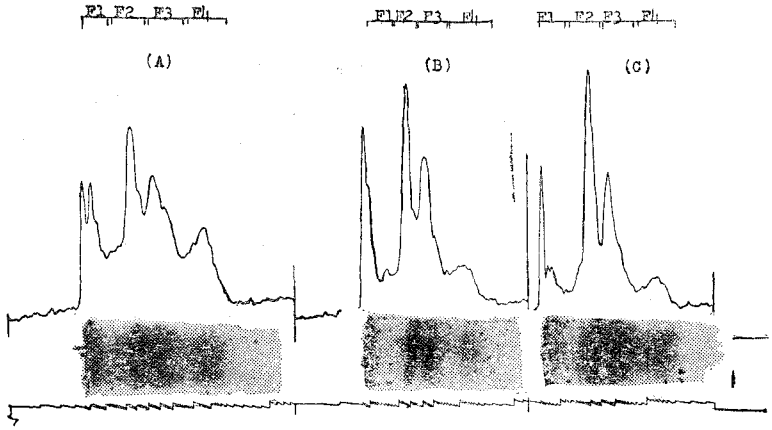



**Fig. 4.** The electropherogram of the extracted RNA from 8-hour-treated wheat coleoptiles. (A), $5\times10^{-5}$ M IAA; (B), $1.5\times10^{-5}$ M ABA; (C) water

**Table 3.** The ratio of each fraction to the total of the high molecular weight RNAs.

| | ratio of each fraction(%) | | | |
|---|---|---|---|---|
| | F1 | F2(25S+23S) | F3(18S+16S) | F4(13S) |
| Control | 12 | 34 | 40 | 14 |
| IAA | 17 | 28 | 33 | 21 |
| ABA | 10 | 30 | 40 | 18 |

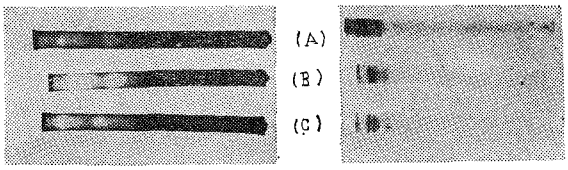



**Fig. 5.** The life-size electropherogram of high molecular RNAs and RNase isozymes. Left; RNase isozymes; Right; RNAs. (A), water; (B), $1.5\times10^{-5}$ M ABA (C), $5\times10^{-5}$ M IAA.

In the total RNA and RNase activity we confirmed the view that IAA increases RNA level and suppresses RNase activity and that ABA does the opposite. The failure in increasing RNase activity in p-hydroxymercury benzoate was in concordance with the view of Barnard (20)

The decrease in r-RNA level caused by ABA treatment may be attributed to the high molecular weight RNA F2. F1 and F4 appear to be respon sible for the enhancement of r-RNA by IAA treatment. The same tendency in both treatments to increase G2 isozyme and G4 isozyme and to decrease G3 and G1 may lead to the assumption that IAA and ABA have some common effects on RNA metabolism, although incubation of each isozyme with the fractionated RNAs is necessary for further elucidation of the exact relation between the isozymes and RNAs.

## Addendum

Upon the completion of the present manuscript the report by Eex (20) was published. Findings by the authors may be in some respects related to the present observation, although attacked subject and the way of approach and interpretation were rather different from those of the paper.

## 요 약

소맥 자엽초를 일정한 길이로 절단하여 식물생장 조절제인 indole acetic acid (IAA)와 abscisic

acid (ABA)를 처리하여 아래와 같은 결과를 얻었다.

1. IAA는 고분자 핵산의 F2와 F3을 감소시킨 반면에 ABA는 F4를 증가 시켰다.

2. IAA는 G2 isozyme의 활성도를 증가시켰으나 G3 isozyme의 활성도는 ABA에 의하여 감소되었다.

3. 상기의 결과로 부터 IAA와 ABA는 핵산과 RNase에 대하여 어느정도 공통되는 효과를 갖는다는 것이 암시됨을 알수 있었다.

4. SH group을 저해하는 pb, p-hydroxymercury benzoate 등에 의해서 발현되는 latent RNase의 활성도는 나타나지 않았다.

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